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Evaluating surveillance strategies for bovine tuberculosis in Scotland

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THE UNIVERSITY
of EDINBURGH

Thesis submitted in fulfilment of
the requirements for the degree of
Doctor of Philosophy
to the
University of Edinburgh — September 2016

Declaration of Authorship

I, Siben Li, declare that this thesis titled, ‘Evaluating surveillance strategies for bovine tuberculosis in Scotland’ has been composed solely by myself and that it has not been submitted, either in whole or in part, in any previous application for a degree or any other professional qualification.

I also confirm that except where otherwise acknowledged, the work presented is entirely my own.

A handwritten signature in black ink, appearing to be 'Siben Li', enclosed within a large, loopy oval shape.

Siben Li
Edinburgh, September 2016

*To my son Sebastian (please do not do a PhD)
wife Jiapeng and dear friend Youyan
without whom this thesis would have
been completed two years earlier*

Lay summary

Bovine tuberculosis (bTB) is a slow spreading disease with serious consequences for Britain's cattle industry. Despite an intensive and costly control programme, the incidence of bTB in large parts of England and Wales is increasing with an exponential rise in cases year on year. During this period, Scotland has consistently reported few cases and was granted status as an officially bovine TB-free (OTF) region in 2009 for the purposes of cattle trading. However, in order to retain its OTF status Scotland must continue to report few cases whilst maintaining its vigilance in detecting potential new cases, as well as minimising expenditure on costly disease surveillance programmes. Prevention and control of bTB is important but challenging, due to the long timescales associated with the disease, the ambiguous transmission routes, the lack of affordable and accurate diagnostic tests, and the effect of complex and changing control policies. With the availability of high-resolution data from cattle movements and the national bTB control programme, there is unprecedented opportunity to study how the disease can spread within, and between farms, and to evaluate the long-term impact of different control strategies.

This thesis has shown that there is variation in the rate of disease-spread within-herds and it is dependent on the herd size and disease duration. On a multi-herd level, in the absence of active regular herd testing, checking animal

carcasses at slaughterhouse for evidence of infection alone is not sufficient to maintain the disease at low and consistent level. Whilst applying more frequent routine herd tests can reduce the overall disease incidence, the performance of the diagnostic test limits the number of detections of truly infected animals and is a big barrier in preventing disease eradication. The primary diagnostic test, commonly known as the skin test, relies on observing an immune response from injected animals; its performance is affected by a number of factors such as time from infection, history of bTB tests on the farm and farm management conditions. I demonstrated by using traditional risk factor analysis that the recent life history of animals such as handling, testing, movement and calving may affect skin test response and this may be partially responsible for the poor test performance. These factors are commonly associated with physiological stress, and there are experimental studies to suggest that stress can depress immune response in animals infected with bTB. However, there are still significant gaps in our knowledge of the complex interplay between physiological stress, disease susceptibility and host immunity. There is a strong need for further research into factors that can affect immune responses in bTB infected animals to better inform traditional epidemiological models, and for developing more cost-effective strategies for disease control and prevention at the industry level.

Abstract

Bovine tuberculosis (bTB) is one of the most complex, persistent and controversial problems facing the British cattle industry. It is also potentially zoonotic and so has public health implications. The incidence of the disease has been increasing in Great Britain for more than 20 years and is now endemic in southwest regions of the country and occurs sporadically elsewhere. Scotland records very few incidences of bTB and was declared as an Officially bTB free (OTF) region in 2009 for the purposes of cattle trading. However, in order to retain its OTF status Scotland must continue to demonstrate the ability to report low level of disease prevalence whilst maintaining its vigilance to potential new outbreaks. This thesis uses a variety of epidemiological and statistical models to evaluate the ongoing control strategies for bTB in Scottish cattle herds and highlight potential limitations to the current surveillance programmes.

In the absence of an established wildlife reservoir, livestock movements are considered the primary mechanism for introduction of bTB into cattle herds. I use movement and bTB data to estimate the within-herd incidence rate for each infected farm in Scotland. The results suggest that this rate varies across farms, and is dependent on the herd size and length of disease exposure. These incidence rates are then used to parameterise a multi-herd dynamic model using stochastic simulations that incorporate multiple disease transmission pathways. With this

approach I evaluate the impact of different routine test protocols on the overall simulated epidemics. Based on the model outcome, abattoir surveillance alone is not sufficient to maintain infection at a low constant level. Whilst adapting to more frequent routine testing regime can reduce disease incidence, the sensitivity of the surveillance methods can also have a big impact on the long term stability of the disease prevalence and can act as the main barrier to eradicating the disease from low incidence regions.

The single intra-dermal comparative cervical tuberculin (SICCT) test used in the current routine herd surveillance relies on stimulating an immune response and observing delayed hypersensitivity reactions in infected animals. The test suffers highly variable, and often poor, sensitivity with current estimates ranging from 50% to 80%. The lower sensitivities may be associated with early stages of infection, concurrent illness, and farm management conditions as well as the presence of sub-clinically infected carriers that can potentially escape detection. In addition, there was evidence that physiological stress can have a marked effect on the immune responses in animals affected with bTB. I conducted two different types of case-control analyses to investigate the potential effect of stress related events on the outcome of the SICCT test.

In the first analysis, a matched design is implemented to examine the effect of recent calving on reactivity to the SICCT. SICCT test positive cattle (cases) were matched with test negative (control) animals within the same farm. By selecting herd-mates (i.e. animals within the same herd at the same time), the study aims to control for space and time. Furthermore, animal age and breed were used as additional selection criteria to control for previous exposure period and potential genetic variation to the reaction of SICCT test outcome. Results from a conditional logistic regression model indicated that animals calved within 60 days prior to test were less likely to respond to the SICCT test in comparison

to non-recently calved animals, and that this effect was strongest in the first 2 weeks of the post-partum period.

In the second analysis, animals identified with gross pathology at *post-mortem* (TB-like lesion and/or bacteria culture) and that were SICCT test negative within 60 days prior to slaughter (representing false negative) were compared with confirmed test positives (true positives). Results from multivariable logistic regression model suggested that the probability of missed infection by SICCT test increases with age and male cattle have higher odds of being a false negative compared to females. Repeated skin tests within 60 and 120 days, as well as recent movement and parturition, were all statistically associated with false negative test outcome. Under future surveillance systems, these results could be used to adjust the timings of testing relative to calving, movements and previous test occasions in order to minimise the risks of false negative test results. Alternatively, increasing the threshold for reactor definition in animals under these categories could be considered to complement the poor test sensitivity.

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Chapter 1

General Introduction

1.1 Bovine tuberculosis

Bovine tuberculosis (bTB) is a chronic, contagious respiratory disease caused by bacteria, *Mycobacterium bovis*, which is closely related to the bacilli that cause human and avian tuberculosis (Smith *et al.*, 2006a). This disease can affect practically all mammals, causing a general state of illness, coughing and occasionally, eventual death (OIE, 2009). It is also a significant zoonotic pathogen particularly in developing countries where surveillance and control activities are often inadequate or unavailable (Cosivi *et al.*, 1998). Worldwide, *M. bovis* accounts for 3.1% of human tuberculosis, however in developing countries such as sub-Saharan Africa, this can be as high as 30% (Müller *et al.*, 2013)

In countries with advanced test and control programmes bTB is a low incidence infectious disease with an apparently low transmission rate in cattle (Skuce *et al.*, 2011). Although the infection level has been controlled in most developed countries, the complete elimination of the disease is complicated by

persistent infection of wild animals such as badgers in the United Kingdom (UK) and Ireland (Donnelly *et al.*, 2003; Byrne *et al.*, 2015), white tailed deer in parts of the USA (United States of America) (Norby *et al.*, 2004) and brush-tail possum in New Zealand (Collins *et al.*, 1986), causing major difficulties in control and surveillance activity and presenting great challenges to local veterinary authorities.

Currently, the disease occurs globally and it appears to be an ever-increasing problem worldwide with significant implications for both animal and human health causing major economic consequences for animal production and public health, particularly in developing countries (Humblet *et al.*, 2009).

1.1.1 *Mycobacterium bovis*

Mycobacterium bovis is a member of the *Mycobacterium tuberculosis* complex, which also includes six other species and subspecies: *M. tuberculosis*, *M. canettii*, *M. africanum*, *M. pinnipedii*, *M. microti* and *M. caprae* (Smith *et al.*, 2006a). It should be noted that *M. caprae* and *M. pinnipedii* were previously considered to be *M. bovis* having identical 16s RNA sequences and with over 99.9% identity of their genome sequences (Cousins *et al.*, 2003; Aranaz *et al.*, 2003). In particular, though less common, *M. caprae* has been identified as causal agent of bTB in central Europe with disease manifestation not considered to be substantially different from that caused by *M. bovis* and the same test can be used for its diagnosis (Prodinger *et al.*, 2005; Rodriguez-Campos *et al.*, 2014). The most notable member of the complex is *M. tuberculosis*, the most important bacterial pathogen of humans, infecting one-third of the population worldwide and causing over two million deaths each year (Corbett *et al.*, 2003; WHO., 2002). Like all *M. tuberculosis* complex, the spread of *M. bovis* infection is considered to be a

relatively slow and progressive process (Menzies and Neill, 2000). The variable latent / incubation period means that the disease can take from a few weeks to a lifetime to develop from infection to clinical disease and to become infectious to other hosts (OIE, 2009).

Although commonly defined as a chronic debilitating disease, bovine tuberculosis can occasionally assume a more acute course where clear clinical signs or emaciation and coughing maybe observed (de Lisle *et al.*, 2001). Generally, *M. bovis* is known to persist within granulomas, distinct lesions represented by a caseonecrotic core surrounded by epithelioid macrophages, T cells, B cells, Langhans-type multi-nucleated giant cells, and fibroblasts (Palmer *et al.*, 2007). Though any body tissue can be affected, characteristic tuberculous lesions occur most frequently in the lymph nodes (particularly of the head and thorax), lungs, intestines, liver, spleen, pleura, and peritoneum (OIE, 2009). Though in many cases, the course of infection is chronic and clinical signs may be lacking, even in advanced cases when many organs may be involved.

1.1.2 History of disease

The name “Tuberculosis” is derived from the nodules that form inside the lymph nodes of infected hosts called “tubercles” (Anonymous, 2013). In 1881 Robert Koch discovered that the tubercle bacillus is the main cause of tuberculosis, and it was not until 1898, the bacterium *M. bovis* was subsequently identified (OIE, 2009). The first ever reference to the occurrence of tuberculosis in animals was made in South Africa by a veterinary surgeon called Hutcheon dating back to as early as 1880 (Hutcheon, 1880). It was believed that bTB existed in the Mediterranean littoral before the classical times, and it spread from northern Italy to western Europe and Great Britain (Renwick *et al.*, 2007). From there, infected

cattle carried the disease to many parts of the world that had been colonised by Europeans (Myers and Steele, 1969; Renwick *et al.*, 2007).

Human TB became a serious problem in Victorian England as industrialisation crowded people together in insanitary conditions in large cities. At the same time, fresh milk was being consumed from dairy herds that were infected with bTB. This was a potent source of infection for many people, particularly children, many of whom died from consuming infected unpasteurised milk (TB FREE England, 2008). Later studies also confirmed that the human prevalence of bTB showed a close correlation with the level of infection in the cattle population in the country (Cosivi *et al.*, 1998). As a result, pasteurisation of milk was first introduced in 1935, and was made compulsory by law in the UK in the 1960s to protect humans from bTB. It was demonstrated that the process could sufficiently kill the bacteria by heating to specific temperature (without spoiling the product) and this largely eliminated the spread of bTB to people (de la Rua-Domenech *et al.*, 2006; Torgerson and Torgerson, 2010). Also during 1935, the British Government employed the tuberculin skin test as a screening tool, which could identify infected cattle (as “reactors”) before they showed symptoms and most importantly enabled the routine testing of cattle for bTB (Anonymous, 2013). The tuberculin skin test was first developed by a Austrian scientist Clemens von Pirquet in 1907 following the description by Koch and the tuberculin hypersensitivity (Lee and Holzman, 2002).

The test is still used as the main detection mechanism for bTB today. Since it was impracticable during the early 1940s to slaughter reactors as soon as they were identified, the only alternative was to free as many herds as possible under a voluntary scheme so that, at a later date, when the overall incidence of infection had been reduced to manageable levels it would be possible to introduce radical measures to eradicate the disease from all cattle herds. This was seen to have the

potential to eradicate the disease and consequently, the “Attested Herd Scheme” was developed (Defra, 2014b). Farmers in all parts of the country were encouraged to voluntarily eradicate bTB from their herds, so leading to eradication from selected areas in the country and ultimately to free all cattle from disease (Macrae, 1961). Once a certain proportion of the herds were in the voluntary scheme, national compulsory eradication of bTB was rolled out to the rest of the country in 1950. The whole of Great Britain (GB) became “attested” on 1st October 1960 (i.e. each cattle herd was certified as being subject to regular tuberculin testing with immediate slaughter of any reactors, just like what happens today). In addition, many whole herds with high levels of infection or persistent infection were depopulated. These practices progressively reduced the number of reactors in the country, with the incidence of bTB reaching a historical minimum in the late 1970s and early 1980s. The highly successful test and slaughter scheme reduced the annual number and rate of test reactors from nearly 15,000 (16.2 reactors per 10,000 cattle tests) in 1961 to 569 (2.3 reactors per 10,000 tests) in 1982 and bTB had almost disappeared (Anonymous, 2013).

There were periods during the mid 1970s, when all cattle herds in the UK had been cleared of bTB, but unfortunately, not all at the same time (TB FREE England, 2008). Particularly in areas of Cornwall and Gloucestershire where herds that had been cleared of the disease continued to have further outbreaks of confirmed bTB, despite the retention of annual (and occasionally more frequent) tuberculin testing. This situation continued at a low level throughout the 1970s. *M. bovis* was first diagnosed in wild Eurasian badger (*Meles meles*) populations in 1971 on a Gloucestershire farm, and it was suggested to have contributed to the re-current outbreaks of bTB in these hotspot areas (Muirhead and Burns, 1974; Cox *et al.*, 2005). Attention had begun to shift to the badgers as a possible wildlife reservoir of infection. Between 1973 to 1998, the cattle test-and-slaughter regime was complemented with a succession of culling strategies,

aimed at reducing badger populations in endemic areas of bTB, but in the absence of experimental controls it is not possible to know whether the observed fall in herd breakdowns was due to badger removal or some other factors (Krebs, 1997). Following recommendations from a previous study, a large-scale field trial - the Randomised Badger Culling Trial (RBCT) was set up to quantify the impact of culling badgers on incidence of bTB in cattle, and to determine the effectiveness of strategies to reduce the risk of bTB herd breakdown. The RBCT represents nearly 10 years of work (1998-2007) and approximately £50 million of taxpayer investment. The final report published in June 2007 explicitly states that badgers contribute significantly to the disease in cattle and that there is a dynamic cycle of infection between the two species (Bourne, 2007). Though the consequence of failure to remove all infected cattle from some farms were due to a combination of factors including weakness with the testing regime (e.g. poor sensitivity of the skin test), and the need for increased active surveillance as well as the reintroduction of disease by badgers. One of the conclusions reached during early stages of the study was that because not all badgers had been caught after the trial had started, it was therefore not possible to accurately quantify the relative importance of badgers and cattle in transmitting infection (Bourne *et al.*, 1998). However, there is evidence from the RBCT that at least 40-50% of cattle herd breakdowns were due to badgers in high incidence areas (the effect seen in the core of proactively culled areas) (Defra, 2007). Recent studies using genotyping and whole genome sequencing WGS technology provided direct genetic evidence of *M. bovis* persistence on farms with a continued and ongoing interaction with local badgers (Biek *et al.*, 2012). However, despite the unprecedented resolution of WGS data, directionality of transmission cannot yet be inferred, more extensive sampling and analysis will be needed for quantification of the extent and direction of transmission between the two hosts.

Despite efforts in the test-and-slaughter control programme in combination

with strategic culling of badgers (with several temporary halts), the number of cases started to rise again during the late 1980s, with a year on year increase of around 18% in the 1990's (TB FREE England, 2008). The situation became worse after the 2001 Foot and Mouth (FMD) outbreak, when the tuberculin testing was temporarily suspended (Carrique-Mas *et al.*, 2008). It was shown that the cessation of bTB testing and large number of herds restocking from high risk areas during this period has led to an increase in the number of reactors in the following years, rising to a 24% increase in reactor rate and 40,000 test positive animals slaughtered in 2008 across GB (Carrique-Mas *et al.*, 2008; Defra and APHA, 2013). Unfortunately since the 1980s, the increase in number of cases and affected geographical areas meant that bTB in cattle is once again widespread in England and Wales.

Although Scotland achieved official tuberculosis free (OTF) status in 2009, the overall prevalence of bTB infection in GB is currently the highest in Europe. Consequently, the current national cattle testing is more frequent than ever, with more than 9 million individual tests conducted during 2015 and approximately 35,000 cattle slaughtered due to bTB control, with an average herd-level prevalence of around 5% across the country (Defra and APHA, 2013). Due to the persistent high bTB incidence and substantial economic consequences for farmers and government, there have been a number of changes in policy in recent years (shifting towards a risk-based surveillance strategy) and this has led to changes in the legislation, which are still ongoing.

1.1.3 Importance of the disease

Bovine TB is the most pressing animal health problem in the UK, the disease is zoonotic but mainly affects cattle, other species of mammals can

also be infected, though only a handful (including badgers in parts of GB) can actively maintain the disease. The scale of infection and the associated costs (including loss of productivity and disease surveillance) make bTB one of the biggest challenges that the cattle farming industry faces (Defra, 2015a). Despite considerable success in controlling this disease in cattle populations in many developed countries (such as New Zealand (Ryan *et al.*, 2006), Spain (Alvarez *et al.*, 2012) and Australia (Radunz, 2006)), bTB remains a sufficiently important economic problem in others (Gordon, 2008; Torgerson and Torgerson, 2010).

In developing countries from many parts of the world, *M. bovis* also affects farmed and wild animals, with accompanying economic and social consequences (discussed in the following sections).

1.1.3.1 Public health significance of bTB

Mycobacterium bovis is not a major cause of human tuberculosis, which is principally caused by *M. tuberculosis*, but humans are susceptible to *M. bovis*. Humans can be infected both by consumption of raw and contaminated milk from infected cattle, or by inhalation of infective droplets (de la Rua-Domenech *et al.*, 2006). In most developed countries of the world, the disease in farmed animals is now relatively well controlled and supplementary precautions of regulated meat inspection and milk pasteurisation have minimised the risk of human infection from *M. bovis* (Neill *et al.*, 2005). Though recent study suggests that the burden of *M. bovis* might be underestimated in human beings as the cause of zoonotic tuberculosis, particularly in low-income countries with absence of systematic surveillance and high burdens of tuberculosis (Olea-Popelka *et al.*, 2016).

Based on current information available from literature, the estimated occurrence of zoonotic TB caused by *M. bovis* in countries of the developed world is

$< 1\%$ of all tuberculosis cases (Grange, 2001). Although some argue that reporting zoonotic tuberculosis cases as a relative proportion of all tuberculosis cases obscures the fact that even a small proportion of the approximately 10 million estimated tuberculosis cases per year globally (WHO, 2016) still represents a substantial absolute number of zoonotic tuberculosis cases. For example, with use of available data, WHO estimated that in 2015 there were 149,000 new cases of zoonotic tuberculosis of which an estimated 13,400 deaths were due to *M. bovis* globally (WHO, 2016). In such instances, infection is often seen in the elderly, who have agriculture associations, and disease has probably arisen from direct contact with infected animals through reactivation of dormant lesions (Neill *et al.*, 2005). Aside from the farming community, abattoir workers and meat handlers are occupationally amongst those at highest risk. They can potentially contract the disease from aerosols generated through handling infected carcasses (e.g. respiratory) or accidental *M. bovis* inoculation through skin contact. This can result in pulmonary tuberculosis or more severe non-pulmonary manifestations following dissemination (Neill *et al.*, 2005). These are however, rare cases, and there is now greater awareness of zoonotic risks of bTB amongst these occupational groups. Following an extraordinary increase in bTB incidence in recent years, the UK Food Standards Agency has offered reassurance on concerns about meat from tuberculin reacting cattle and highlighted that the public health risk is extremely low (Anonymous, 2002a).

However, definitive statements about the public health risk from bTB in developing countries cannot be made with such confidence. Naturally, the occurrence of zoonotic TB is greatly dependent on the presence of bTB in cattle (Müller *et al.*, 2013). Information on the global distribution and prevalence of bTB is scarce, but available data suggest that bTB in cattle is prevalent in virtually all major livestock-producing countries of the developing world, especially those in Africa (Cosivi *et al.*, 1998; Ayele *et al.*, 2004; Michel *et al.*, 2010). Effective

disease control, including regular milk pasteurisation and slaughterhouse meat inspection is largely absent in these regions (Kelly *et al.*, 2016). Consequently, the majority of the human population is at risk of exposure to bTB. Despite the lack of large-scale, population based data, it is estimated that approximately 3% of all TB cases in humans were caused by *M. bovis* in Africa (Müller *et al.*, 2013). In particular, for regions in Ethiopia, Nigeria and Tanzania, the proportion of TB cases caused by *M. bovis* can be as high as 30% (Shitaye *et al.*, 2007; Müller *et al.*, 2013). This situation is exacerbated by the presence of multiple additional risk factors such as human behaviour and the high prevalence of HIV infections (Cosivi *et al.*, 1998; Ayele *et al.*, 2004). Tuberculosis and HIV infection form a lethal combination, each enhancing the impact of the other. The weakened immune system in the HIV-infected host makes tuberculosis more likely to become active and therefore act as a disease dissemination source (Neill *et al.*, 2005). It has been recognised that data collected from developing countries, mainly from sub-Saharan Africa, are insufficient to represent the real situation and possible underestimation must be considered (Ayele *et al.*, 2004). Given the high prevalence of bTB in many African countries and the close interactions between cattle and human populations, the disease must be considered as representing a potential health hazard both to animals and humans in these regions. Although for developed countries, such as UK and Ireland, bTB is no longer a significant public health problem.

1.1.3.2 Economic importance

Currently, in developed countries, bTB is well controlled or eliminated in most areas, and cases of zoonotic TB are rarely seen (Torgerson and Torgerson, 2010; Michel *et al.*, 2010). With low public health risks, developed countries now tend to emphasise the local and international restrictive trading implications of

bTB, rather than the potential for human infection. The European Community regulations lay down the specific requirements for the trade of cattle in relation to bovine tuberculosis and define the “officially tuberculosis-free bovine herd” (OTF) status (Anonymous, 1964). Intra-community trade in bovine animals for breeding and production purposes can only take place out of such herds. In order to comply with European Union (EU) regulations, the bTB eradication programme in the UK requires routine testing of cattle herds using the SICCT test (Monaghan *et al.*, 1994; Defra, 2008a). Detection of one or more SICCT-test positive animals (“reactors”), or *M. bovis* cultured from lesions detected at the slaughterhouse, triggers a herd “breakdown” (Karolemeas *et al.*, 2011). As a consequence, the OTF status of the herd is either suspended (if suspected bTB cases are identified) or withdrawn (if bTB is confirmed) until two consecutive clear herd tests within 60 days intervals are achieved (Szmaragd *et al.*, 2012).

According to the Department for Environment Food & Rural Affairs (Defra), the estimated average cost of a bTB breakdown on a farm in GB is around £34,000. Of this, it is estimated £20,000 is borne by the government, mainly as compensation for animals compulsorily slaughtered and the costs of testing, £14,000 falls to the farmer as a result of the loss of animals, on-farm costs of testing, and business disruption because of movement restrictions (Defra, 2015b). Statistics in 2011/12 indicate that the average cost of a routine bTB test was £350 to farmers and £770 to taxpayers. Overall, in the last decade, £500 million were spent on bTB control, and based on current expenditure, the forecast cost over the next decade will exceed £1 billion, if no additional interventions were applied (Defra, 2014b).

The absence of zoonotic TB despite an upsurge in the incidence of bTB in the UK sparked a controversy over the large financial expenditures for disease control in cattle (Torgerson and Torgerson, 2010). However, if bTB is left unchecked,

the potential risk and impact on the productivity and capability of the livestock industry threatens the nation's ability to trade and grow international exports into new and emerging markets (Defra, 2014b). In developing countries, such as in sub-Saharan Africa, *M. bovis* not only affects farmed and wild animals, combined with the associated public health risks, the accompanying economic and social consequences are potentially even larger.

1.1.4 Host range

Many of the species and subspecies in the *M.tuberculosis* complex show a specific host association (Smith *et al.*, 2006b). In particular, *M. bovis* is most frequently isolated from domestic cattle, but the unusually extensive host range includes most farmed animals (such as farmed deer, sheep, pig) and wild species (such as buffalo, badgers and possums) (Morris *et al.*, 1994). The zoonotic nature of the disease means that humans can also be infected. However, not all species are equally susceptible to *M. bovis*, the isolation from several hosts probably reflect the spillover of strains into an alternative host population rather than a generalised host adaptation (Goodchild and Clifton-Hadley, 2006).

Currently in the UK, European badgers are known to be a wildlife reservoir of *M. bovis*, and are considered to be the only wildlife maintenance host of the disease (Krebs, 1997). They are able to live for several years while infected, breed successfully and transmit disease to other wild and domestic animals (Krebs, 1997). Other domestic and wildlife species diagnosed with *M. bovis* are considered to be spill-over hosts, acquiring infection from the significant maintenance hosts of badgers and cattle (Delahay *et al.*, 2002; Defra, 2006; Broughan *et al.*, 2013). In broader terms, the risk posed by other infected species will generally depend on the level of disease in the population and the environment, such as location, density

and animal behaviour (Anonymous, 2013). There are several other countries in which bTB is present in the wildlife population (Fitzgerald and Kaneene, 2013). A smaller number have a true wildlife reservoirs, in which the wildlife population can sustain bTB infection on its own, regardless of bTB levels in cattle. For instance, there has been recognition that self-maintaining infection is also present in wildlife hosts - notably badgers in the UK and Republic of Ireland (Griffin *et al.*, 2005; Byrne *et al.*, 2015), brush-tailed possums in New Zealand (Porphyre *et al.*, 2008), wild boar and deer in areas of Spain and France (Vicente *et al.*, 2006; Gortazar *et al.*, 2011; Zanella *et al.*, 2008), as well as bison and buffalo in many North America and African countries (Ayele *et al.*, 2004; de Lisle *et al.*, 2001). The importance of these hosts has been highlighted by the growing realisation that these animals can represent the principal source of infection for both domestic animals and protected wildlife (Morris *et al.*, 1994). *M. bovis* diagnosed from other domestic animals such as farmed deer, pigs, cats and dogs are not uncommon (Wahlström *et al.*, 1998; Cousins, 2001; Zanella *et al.*, 2008; Nugent *et al.*, 2015), but their role in sustaining and transmitting infection is less significant, other than in some isolated cases.

Evidence from countries with a wildlife reservoir of infection (such as Australia, New Zealand, Spain and USA) show that bTB control measures in cattle populations alone are not successful in eradicating the disease (O'Brien *et al.*, 2006; Ryan *et al.*, 2006; Miller and Sweeney, 2013). Control and eradication of bTB therefore requires addressing the disease in both wildlife hosts and domestic cattle with a package of control measures aimed at reducing transmission and the overall burden of bTB infection.

1.1.5 Epidemiology

Bovine TB is primarily a respiratory infection, and spread mainly by direct contact with infected domestic and/or wild animals (Cousins, 2001). Infectious aerosols may originate from sputum (the respiratory tract) or from contaminated fine dust particles, a potential route by which environmental contamination could be rendered infectious (Menzies and Neill, 2000). Based on results in a survey where infected cattle confirmed by laboratory culture were examined, it was found that 20% of animals yielded *M. bovis* from their upper respiratory tracts, these animals are considered to have higher chance of excreting *M. bovis* and potentially contribute to onward infection (Menzies and Neill, 2000). Respiratory transmission appears to require lesions in the lungs and associated lymph nodes. Most (40-73%) confirmed reactors have lung lesions, although many are too small to be detected routinely at abattoir meat inspection (McIlroy *et al.*, 1986a). Calves and humans can also become infected by ingesting raw (unpasteurized) milk from infected cows (Neill *et al.*, 2005). The disease can take a variable amount of time (from a few weeks to a lifetime) to develop from infection to clinical disease and to become infectious to other animals (Anonymous, 2013). Thus the chronic nature of the disease means that it can take from a month to years for the reactivation of latent bTB from a primary infection (Perezill *et al.*, 2011). This latent effect gives a large time frame for the disease to potentially spread undetected within the herd before it begins to manifest clinical signs. Therefore, movement of undetected infected domestic animals and contact with bTB infected wildlife are considered as the major ways of spreading the disease (Green *et al.*, 2008; Donnelly *et al.*, 2006). Though risk factors including biological, behavioural, environmental and genetic are all known to influence both the transmission and susceptibility to *M. bovis* (Skuce *et al.*, 2011).

1.1.5.1 Transmission

In general, the routes of infection identified in pathogenesis studies strongly suggest that bTB is predominantly a respiratory infection (OIE, 2009). Hence, any situation of close and sustained contact directly with an infectious case may facilitate transmission (Phillips *et al.*, 2003). A review of the literature supports the view that *M. bovis* is mostly transmitted via infectious aerosol (Delahay *et al.*, 2002; Courtenay *et al.*, 2006; Flynn *et al.*, 2009). Indirect routes of transmission would include for example, a contaminated external or internal environment (in-relation to herd), contaminated feed, water or equipment (Courtenay *et al.*, 2006; Fine *et al.*, 2011). However, on balance, direct contact would seem to be more significant than transmission potentially supported by ‘indirect’ routes (Böhm *et al.*, 2009; Fine *et al.*, 2011). In addition, several studies demonstrated evidence of ‘direct’ infection from dam to calf either congenitally or from ingestion of tuberculous milk. This is supported by pathogenesis data, where pathology differing from the classical respiratory tract were observed, which suggests ingestion of *M. bovis* (O’Reilly and Daborn, 1995; Phillips *et al.*, 2003; Serrano-Moreno *et al.*, 2008).

The main (hypothesised) pathway of cattle-to-cattle transmission of bTB have been summarised by (Skuce *et al.*, 2011). They are listed below in no priority order.

- Within-herd transmission at housing and pasture
- Vertical (congenital) transmission or pseudo transmission via contaminated milk
- Between-herd transmission through cattle movement
- Between-herd transmission across farm boundaries (airborne)

- Contamination from grass, soil and silage (*M. bovis* may be excreted or secreted onto grass or soil from cattle or wildlife saliva, nasal secretions, urine or faeces. The bacteria probably remain viable and pathogenic in soil for about 6 months (Phillips *et al.*, 2003). Contaminated pasture or soil can transmit infection via ingestion by cattle).
- Contaminated drinking water (Michel *et al.* (2007) has demonstrated that shedding of *M. bovis* in nasal and oral secretions may lead to contamination of ground or surface water and subsequent transmission to other species)

As well as cattle-cattle transmission, the situation of bTB is complicated by the existence of infected wildlife hosts. Molecular studies on bTB reveals a striking pattern of geographic clustering of *M. bovis* genotypes at a local level in the UK, while marked geographical localisation of *M. bovis* genotypes has also been reported in Northern Ireland (Gopal *et al.*, 2006; Smith *et al.*, 2006a; Skuce *et al.*, 2010). This phenomenon may reflect the opportunities for cattle-cattle transmission supported by the natural and imposed contact networks and movement restrictions imposed on cattle, though another plausible hypothesis suggests that this regional clustering simply reflects the underlying structure of infectious wildlife (Gilbert *et al.*, 2005; Smith *et al.*, 2006a; Defra, 2008b). Furthermore, molecular typing data showing that cattle and badger populations shared the same genotypes within localised geographical clusters provides further evidence to this claim. The extent to which bTB is self-sustaining in either cattle or wildlife (badger) populations alone is of crucial importance to disease control. Whilst it is widely accepted that infectious badgers contribute significantly to the epidemiology of bTB in the British Isles (Donnelly and Hone, 2010; Byrne *et al.*, 2015), some argue that the opportunities for the disease to be transmitted via cattle-cattle are actually greater than those for the transfer of infection between badgers-cattle (Bourne, 2007). Though in many instances, it has proven to be more difficult to control or eradicate bTB in wild free-ranging species than in

domesticated cattle (Fitzgerald and Kaneene, 2013). A recent study on patterns of direct and indirect contact between cattle and badgers naturally infected with bTB has shed some light on the way that the disease is transmitted on pasture (Drewe *et al.*, 2013). However, due to the complex interactions of cattle population and wildlife hosts (i.e. badgers in the UK), it is still not clear whom is infecting whom and more investigations are needed to determine how the transmission actually occurs between them (Skuce *et al.*, 2011). The complex biology of the pathogen and the ambiguity of the transmission pathway make the study of bTB epidemiology particularly challenging.

1.1.5.2 Susceptibility

In epidemiology, a susceptible individual is a member of a population who is at risk of becoming infected by a disease (Thrusfield, 2007). There is relatively limited knowledge about the factors which influence susceptibility / resistance to bTB and this is an obvious blind-spot in the knowledge and evidence base, especially locally (Skuce *et al.*, 2011). But generally speaking, disease susceptibility and transmission are intimately linked and some or all of the same risk factors (genetic and non-genetic) may influence both (Morens *et al.*, 2004). Genetics research aims to find out the extent to which differences in susceptibility (or resistance) are due to the genetic makeup of animals and then to find the gene variations responsible for those differences. For example, on-farm studies in Ethiopia suggest Holstein cattle showed higher skin test prevalence and disease severity compared to their zebu herd-mates (Vordermeier *et al.*, 2012), while other findings have also demonstrated significant heritability in prevalence of incidence to bTB in Holstein cattle in the Republic of Ireland (ROI) (Bermingham *et al.*, 2009) and in the UK (Brotherstone *et al.*, 2010). While it is biologically plausible to assume that genetic variation in both the host and the pathogen has

potential to influence the outcome of exposure, detection, infection, disease and infectivity. The heritability shown in susceptibility to disease and the outcome of bTB diagnostic test indicates that there is exploitable genetic variation in risk (Allen *et al.*, 2010). It is therefore possible to improve the bTB resistance of the national herd by selective breeding and to better understand the genetic variation that underpins disease susceptibility and resistance (Trinkel *et al.*, 2011).

On the other hand, a list of non-genetic risk factors (at animal-level) which may influence bTB susceptibility include animal age (Pollock and Neill, 2002), gender (Wolfe *et al.*, 2009), breed (Elias *et al.*, 2008), concurrent diseases (Flynn *et al.*, 2009), physiological state (Goodchild and Clifton-Hadley, 2001) and immune suppression (Dhabhar and McEwen, 1997). Numerous studies in various countries identify age as a significant animal-level risk factor (Brooks-Pollock *et al.*, 2013; Wolfe *et al.*, 2009; O'Hagan *et al.*, 2015). Several studies have shown that older animals are more likely to have been exposed than younger ones, given the biologically plausible assumption that the duration of exposure increases cumulatively with age (Kazwala *et al.*, 2001; Cleaveland *et al.*, 2007; Inangolet *et al.*, 2008). Therefore, older cattle may be more at risk and should be given particular attention. In addition, age may also affect the probability that an animal tests positive. However in a study that explores age-dependent patterns of bTB (Brooks-Pollock *et al.*, 2013), age-specific incidence increased monotonically until 24-36 months, with cattle aged between 12 and 36 months experiencing the highest rates of incidence. Though the incidence rate of older reactors (more than 3 years) were very similar.

Gender was not a significant risk in ROI studies of reactor herds (Clegg *et al.*, 2008; Wolfe *et al.*, 2009; O'Hagan *et al.*, 2015) but was mentioned as a risk factor in studies carried out in Africa (Kazwala *et al.*, 2001). Relative differences in susceptibility may be masked by differences in longevity of beef and

dairy cattle and the different between- and within-herd movements and contacts experienced by both genders (Alvarez *et al.*, 2009; Bell *et al.*, 2010). Males (Bulls) have potentially more contact with other herds during breeding, which may increase their risk (Humblet *et al.*, 2009). Studies performed in Africa also identified the animal breed as a risk factor, where European breeds may be less resistant than the indigenous breeds such as zebu (Omer *et al.*, 2001). Though the apparent differences between breeds may be more influenced and better explained by different management practices (Elias *et al.*, 2008). Another study using national surveillance data from GB cattle herds tested the possibility that certain breeds (associated with specific genotype marker) does not confer resistance but instead causes cattle to react less strongly to the prescribed diagnostic test (Amos *et al.*, 2013). This study demonstrated a strong association between breed and the SICCT test outcomes, with smaller reactions in the common dairy breeds Jersey, Friesian and Holstein, and larger reactions in various beef breeds and their crossbreds. In contrast, a more recent study by (Tsairidou *et al.*, 2016) concluded that the continuous variation in SICCT outcome is only lowly heritable and has a weak correlation with test positivity among healthy animals which was not significantly different from zero ($p > 0.05$).

Immune-suppression, a significant predisposing risk factor in many diseases of man and animals, is another influential factor in bTB susceptibility (Humblet *et al.*, 2009). Thus, an animal that is immune-suppressed would be more susceptible to infection and disease (Menzies and Neill, 2000). There are many factors which can cause immune-suppression in cattle such as concurrent infection with immune-suppressive viruses, parasites or other mycobacteria (de la Rua-Domenech *et al.*, 2006; Flynn *et al.*, 2009), physiological and nutritional stress from handling, testing, movement, calving and lactations (Buddle *et al.*, 1994; Burton *et al.*, 2003; Clegg *et al.*, 2008; Griffin *et al.*, 1993; Verbrughe *et al.*, 2012).

Other factors potentially influencing susceptibility to bTB include cattle behaviour, climate and weather conditions as well as herd size, type and farm management practices (White *et al.*, 2008; Wint *et al.*, 2002; Thornton, 2010; Skuce *et al.*, 2012). For instance, a modelling study funded by Defra (project SE3003) showed that dairy herds have a higher ‘transmission coefficient’ and are more likely to have a bTB breakdown than beef herds, this may be due to their longevity and more intensive management system, which often results in closer confinement, though there is a general lack of direct biological evidence to the claim (Defra, 2000). Weather and climate has been linked to geographical and temporal variation in bTB, King *et al.* (1999) suggests that if infection was more likely to occur at pasture than indoors, cattle would be infected in early summer and could transmit to the following winter, leading to high numbers of infected animals being detected by beginning of the following year. Though the seasonal effects are probably obscured by the timing of intense bTB testing (i.e. more tests were conducted in autumn and winter months where animals are housed in doors). In summary, there are different levels at which susceptibility and resistance act, for example resistance to infection, resistance to disease, resistance to disease progression and onward transmission/excretion etc (Skuce *et al.*, 2011). From existing literature and reviews, it is difficult to rank the appropriate and identified risk factors for susceptibility (largely due to limitation to population-specific studies). Though experience suggests that if susceptibility to infection can be reduced this would be reflected in reduced infectiousness and reduced onward transmission (Skuce *et al.*, 2011; Goodchild and Clifton-Hadley, 2001).

1.1.6 Diagnosis

The diagnosis of bovine tuberculosis, like human tuberculosis, remains extremely challenging. There is currently no single test which will fulfil all the

criteria necessary to identify all infected animals (Strain *et al.*, 2011). Currently, in GB and internationally, the most commonly used control strategies for detection of bTB is dependent on variants of the tuberculin skin test, combined with slaughter of test positive animals. Other measures include additional examination of animal carcasses for evidence of TB-like lesion and confirmation of infection through bacterial culture (de la Rua-Domenech *et al.*, 2006; Conlan *et al.*, 2012).

But in general terms, the methods for the diagnosis of tuberculosis in cattle can be divided into two broad categories, direct and indirect tests. Direct tests are those that are designed to directly identify the organism in the host animal. Primarily this relates to the *post-mortem* examination of animals and the associated tests used to confirm infection (i.e. identification of lesion and bacterial culture). Indirect tests (*ante-mortem*) are those that identify infection in live animals using indirect indicators of infection. There are of course considerable overlap in how the various tests are implemented. For example, most *post-mortem* histopathology and culture is carried out on animals giving positive reactions using an immunological test or with visible lesions at routine slaughter. Details of the current diagnostic tests under each category are described in sections below.

1.1.6.1 *Ante-mortem tests*

This category of test is largely based upon immunological markers, i.e. those that identify and measure immune responses in the animal to the *M. bovis* organism (Schiller *et al.*, 2010a). Broadly speaking they fall into two categories, those that are based upon the cellular immune response (primarily the intradermal tuberculin skin test and the gamma-interferon test) and those based upon antibody responses.

Skin Test

The skin tests are the international standard for *ante-mortem* diagnosis of bTB and is one of two currently approved tests within the European Union (EU) (de la Rua-Domenech *et al.*, 2006). They are based on eliciting a delayed-type hypersensitivity response to the intradermal injection of tuberculin (a crude protein extract from supernatants of mycobacterial cultures) (Monaghan *et al.*, 1994; Kaufmann and Schaible, 2005). When bovine tuberculin is injected into the skin of an animal not sensitised to tuberculin antigens, there is no significant local response. However, if tuberculin is injected into an animal whose immune system has been sensitised by infection with *M. bovis* or by exposure to cross-reacting antigens, it triggers an inflammatory response and swelling at the injection site that reaches its greatest intensity 48 - 72 hours post-injection and regresses rapidly thereafter (Lepper and Pearson, 1977; Francis *et al.*, 1978; Pollock *et al.*, 2003). This delayed-type hypersensitivity reaction to the intradermal injection of tuberculin is mediated by a population of sensitised T-cells and takes at least some weeks to develop after infection (Francis, 1947).

In its simplest form, the skin test involves the intradermal injection of bovine tuberculin into the skin and measuring the subsequent swelling (for evidence of inflammation) at the site of injection 72 hours later (Francis *et al.*, 1978). The injection is typically performed either in the neck or caudal tail fold, but in general, the skin of the neck is regarded as more sensitive (OIE, 2009). There are now two broad skin test formats used across the world, a single intradermal skin test using *M. bovis* tuberculin alone and the comparative test using *M. avium* and *M. bovis* tuberculins (Strain *et al.*, 2011). The single intradermal comparative tuberculin test (often referred to as SICCT test) is used to differentiate between animals infected with *M. bovis* and those responding to bovine tuberculin as a result of exposure to other mycobacteria (Pollock *et al.*, 2003). According to guidelines

from the national eradication programs in the UK, an animal is classified as a standard reactor for SICCT (test positive) if the increase in skin thickness at the bovine site of injection is 4 mm or more than the reaction from the avian injection (Downs *et al.*, 2013). The reaction is considered to be inconclusive if the reaction to bovine tuberculin is from 1 to 4 mm greater than the avian reaction. This interpretation scheme is used in EU countries and is recommended in Council Directive 64/432/EEC (EU, 1980). Sometimes a more stringent interpretation is used. For example, severe interpretation of the SICCT test is normally applied during Short Interval Tests (SITs) on herds with recent confirmation of bTB, or where tests read at standard interpretation require reinterpretation after reactors with confirmed tuberculosis are revealed. Under severe interpretation, an animal is classified as a reactor if after 72 hours, the reaction to bovine tuberculin is positive and the reaction to avian tuberculin is negative, or animals show a positive bovine reaction more than 2 mm greater than a positive avian reaction (Skuce *et al.*, 2012).

There appears to be a strong association between the degree of disease progression and the magnitude of the SICCT test response (Norby *et al.*, 2004). Moreover, cattle with large skin responses to tuberculin are much more likely to have visible pathology at *post-mortem* than those with more moderate skin responses (Clifton-Hadley and Goodchild, 2005). While it is generally accepted that recently infected cattle may fail to respond to the intradermal skin test (Monaghan *et al.*, 1994), there is uncertainty of how long this period lasts. Experimental work where cattle were artificially inoculated with moderate infective doses of *M. bovis* tested positive within 3 weeks of infection, which suggest the latent period may not be very long (Thom *et al.*, 2006). According to the official OIE information, the delayed hypersensitivity reaction may not develop for a period of 3-6 weeks following infection (OIE, 2009). Thus, if a herd/animal is suspected to have been in recent contact with infected animals,

delayed testing should be considered in order to reduce the probability of false-negatives (i.e. miss diagnose of infection). At the other extreme it has been long recognised that chronically infected animals with severe pathology (i.e. advanced disease) may also become non-responsive to the SICCT test, the so-called anergic animals, though in countries with frequent testing, this is unlikely to be seen (Lepper and Pearson, 1977; Pollock and Neill, 2002).

In practice, herds with disclosed reactor animals are subject to subsequent retesting in order to identify newly infected animals and previously missed infections. However, it appears that the interval between repeated tests may have an important effect on the sensitivity of the SICCT test. This desensitisation effect has been well documented (Radunz and Lepper, 1985; Thom *et al.*, 2004; Coad *et al.*, 2010). Infected cattle previously skin tested for bTB can fail to react if the test is repeated shortly afterwards and it appears that this effect is most intense in the week immediately following the previous test (Doherty *et al.*, 1995). Some studies suggest that this desensitisation largely subsides after 60 days (Thom *et al.*, 2006; Radunz and Lepper, 1985), while others have found that complete recovery of reactivity in the skin test may not be achieved even after 60 days (Doherty *et al.*, 1995). Recent work indicates that repeated intradermal testing at 60 day intervals can lead to increased desensitisation at subsequent tests and this effect is seen both in experimental infections and naturally infected animals (Coad *et al.*, 2010; Thom *et al.*, 2004). This observation may be particularly important for animals testing inconclusive and undergoing repeated tests, since these animals are likely to have marginal SICCT test responses which may be further depressed due to repeated testing and thus, prevent their subsequent disclosure.

Due to many confounding factors (such as those described above) that can potentially affect the performance of the SICCT test, estimating the test

sensitivity has proven to be very challenging. Any estimate will be based upon the specific animals under investigation and may not necessarily be representative of the actual population as a whole. For example, an estimation of the test sensitivity in one area/country may not reflect the situation in a different one (different confounders, genetic make up, husbandry practices etc.) (Strain *et al.*, 2011). Generally speaking, in order to accurately assess sensitivity all animals tested should be slaughtered and subjected to a thorough and accurate test to determine the actual disease status of each animal. In reality rarely have all tested animals been slaughtered (most studies were based on slaughtered reactors only) and the *post-mortem* examinations thorough enough to have a high level of certainty of the actual disease status but again is not 100% accurate. Many studies have tended to be small, selecting animals from high prevalence herds (de la Rua-Domenech *et al.*, 2006). In current literature, there are a range of estimates for the SICCT test sensitivity ranging from 51% to 89% (Mitchell *et al.*, 2006; Downs *et al.*, 2011; Clegg *et al.*, 2011a) under the standard interpretation. Sensitivity increases when the interpretation is severe but at the expense of lower specificity. One report suggested that the sensitivity of the test could be increased from 83% to 93% if the test interpretation is changed from standard to severe (de la Rua-Domenech *et al.*, 2006). More recent modelling work has estimated the sensitivity (Se) and specificity (Sp) ranges for standard and severe interpretations of the SICCT test to be 70 - 89% (Se standard) and 78 - 91% (Se Severe), $99.98 \pm 0.004\%$ (Sp Standard) and $99.91 \pm 0.013\%$ (Sp Severe) (Karolemeas *et al.*, 2012; Goodchild *et al.*, 2015). It is worth noting however, that these values were all considered to be “relative” estimates as they were based on comparisons with a baseline scenario. While most sensitivity estimates are made at the animal level, the herd level sensitivity will be inherently higher even when the within herd prevalence is low (de la Rua-Domenech *et al.*, 2006). This opens up the possibility of employing ancillary tests with greater individual animal sensitivity in herds with confirmed

infection even if the ancillary test has a reduced specificity. This could include for example the use of gamma-interferon blood assay as described below.

Gamma-interferon assay

The gamma-interferon blood assay is an alternative test approved under the EU directive 64/432 annex B as an ancillary test for bTB (EU, 1980) and is also an OIE listed test for the purpose of international trade (OIE, 2009). The test measures the cellular response (in the form of release of gamma-interferon) from sensitised blood lymphocytes exposed to mycobacterial antigens using a sandwich ELISA (Wood *et al.*, 1990). Immune cells present in the blood respond to the antigens releasing a cascade of chemical signals (immune responses - most notably the activation of macrophages) (Strain *et al.*, 2011). A positive result is defined when there is detectable level of gamma-interferon (IFN- γ) above a background in the sample to constituents of *M. bovis* compared to other mycobacteria (Wood and Jones, 2001). The antigens currently used in GB and Ireland are purified protein derivatives from *M. avium* (PPDa) and *M. bovis* (PPDb) and putative TB complex specific antigens such as ESAT-6 and CFP-10 (which is suggested to have higher sensitivity and specificity) (OIE, 2009). It is currently marketed as Bovigam[®] (Prionics, Switzerland).

The gamma-interferon test is capable of detecting early stage infections (3-5 weeks following infection even when the level of exposure is very modest (Dean *et al.*, 2005)), and when used in parallel to the SICCT tests, allows detection of a greater number of infected animals before they become a source of infection for other animals (Gormley *et al.*, 2006; Lahuerta-Marin *et al.*, 2016). The advantage of the gamma-interferon test over the SICCT test is that the animals need be handled only once and the test interpretation can be regarded as less subjective (de la Rua-Domenech *et al.*, 2006). It also has a better sensitivity (when compared with the SICCT test under standard interpretation) with estimates ranging from

73% (Lilenbaum *et al.*, 1999) through to 100% (median: 87.6%) (Monaghan *et al.*, 1997). While test specificity were significantly lower with estimates range from 85% (Buddle *et al.*, 2001) to 99.6% (median: 96.6%) (de la Rua-Domenech *et al.*, 2006). In a recent meta-analysis of studies, sensitivity and specificity estimates of 86.19% and 96.63% were made (compared to 51.11% and 99.58% for the SICCT under standard interpretation) (Downs *et al.*, 2011). Again, these figures are “relative” estimates and caution needs to be taken during their interpretations. Broadly speaking, it would seem that the gamma-interferon test has the ability to detect a slightly different subset of infected animals (ones that are less responsive in the SICCT test), therefore the application of both tests often resulted in an increased sensitivity. Other epidemiological studies have demonstrated that the ancillary use of gamma-interferon test (in parallel to SICCT) can reduce the proportion of false negative outcome (i.e. non-reactor cases) from the SICCT (Alvarez *et al.*, 2014; Lahuerta-Marin *et al.*, 2016, 2015).

In addition, unlike the SICCT test, which can potentially suffer from desensitisation due to repeated testing within short periods of time, there was no evidence of this depressing effect in the gamma-interferon test (Doherty *et al.*, 1995; Buddle *et al.*, 1994). However, interestingly there is some controversy over the effect of prior intradermal skin testing on subsequent gamma-interferon testing in cattle. Early work suggested there might be a transient depression in the gamma-interferon responses of cattle following intradermal skin testing, although this was only observed in 2 out of 4 experimentally infected cattle (Rothel *et al.*, 1992). A similar effect was seen in experimentally infected cattle tested 3 days after a SICCT (Whelan *et al.*, 2004), although in contrast, this effect was not present in naturally infected cattle (Coad *et al.*, 2007). Another study found that intradermal testing actually boosted the gamma-interferon responses in blood samples for several weeks with no evidence of detrimental effect on the test results (Whipple *et al.*, 2001). Similar studies in both the UK and Ireland also concluded

that the gamma-interferon test could reliably be used following repeated SICCT testing on naturally infected cattle (Doherty *et al.*, 1995; Gormley *et al.*, 2004; Thom *et al.*, 2004). Furthermore, findings from a Northern Irish study suggest that gamma-interferon positive animals represent a higher risk of failing a SICCT test in the future, indicating the value of gamma-interferon testing for identifying early-stage infections (Lahuerta-Marin *et al.*, 2015).

Although gamma-interferon offers several practical advantages over the tuberculin skin tests, due to the high costs and the more complex nature of laboratory-based assays (culture start is required within 24h after blood sampling), as well as the limitation of reduced specificity (i.e. identification of unacceptably high number of false positive animals), they are usually used as ancillary tests to maximise the detection of infected animals (parallel testing), or to confirm or negate the results of an intradermal skin test (OIE, 2009; Strain *et al.*, 2011). Though it may well be acceptable in some circumstances to apply the gamma-interferon test as a primary detection tool, however, the usefulness and cost effectiveness of the test either used on its own or in combination with the intradermal skin tests in controlling the disease spread is still not clear (Vordermeier *et al.*, 2005). It was suggested that more attention should be focused on improving the properties of the gamma-interferon test, especially its specificity (Coad *et al.*, 2007). Some studies recommend that in order to maximise the impact of this test on disease outcomes an alternative targeted approaches needs to be adapted rather than using it in a wholesale way in multiple reactor herds (Anonymous, 2002b).

It is worth highlighting that the accuracy of the diagnostic test (both tuberculin skin test and gamma-interferon) to identify tuberculosis infected animals is almost certainly confounded by a number of factors such as, co-infection (e.g. parasitism), masking infection (e.g. *M. avium paratuberculosis*), nutritional

and physiological stress (e.g. pre and post-parturition period), inadequate testing techniques and drug interactions (e.g. dexamethasone therapy) (de la Rua-Domenech *et al.*, 2006; Shitaye *et al.*, 2007; Strain *et al.*, 2011). For example, studies have found increasing evidence that co-infection with other pathogens affects the diagnosis of bTB, such as co-infection with Johne’s disease (i.e. *M. bovis* and *M. avium paratuberculosis*) can significantly reduce the sensitivity of gamma-interferon test (Alvarez *et al.*, 2009). Similar work on investigating co-infection with *Fasciola hepatica* (liver fluke) and *M. bovis* on bTB diagnostics has also found depressed response under both the gamma-interferon and comparative skin test compared to *M. bovis* alone infected cattle (Flynn *et al.*, 2009; Claridge *et al.*, 2012). Other earlier studies indicated that infected cattle failing to respond to the skin test immediately following parturition but skin tested positive 4-6 weeks later (Kerr *et al.*, 1946), while Buddle *et al.* (1994) also observed a suppressed immune response under gamma-interferon test in periods immediately post-calving in comparison to responses at pre-calving level in experimentally infected cattle. In addition, both types of tests share the disadvantage of low probability of detecting infected cattle in a state of depressed cell-mediated immune response to tuberculin (in the form of ‘anergy’), although no data are available on the actual numbers in bTB prevalence areas (de la Rua-Domenech *et al.*, 2006). Furthermore, there is evidence to suggest that animal-level and herd-level factors may also effect the ability for diagnostic test to identify infected animals

Given the accumulating body of evidence suggesting that various extrinsic factors are likely to influence the diagnostic outcomes for bTB and possibly the disease transmission dynamics, more attention should be focused on investigating and identifying these effects.

Antibody tests

Due to the nature of bTB infection, the development of an accurate antibody-based test has been particularly arduous and limited (Pollock and Neill, 2002). Antibody responses to *Mycobacteria tuberculosis* complex organisms are generally regarded as muted in most animals with the predominant immune response being cellular (Strain *et al.*, 2011). Classically following infection, animals elicit an early and robust cell mediated immune response which can at times, shift towards an antibody-based response as the disease progresses (de la Rua-Domenech *et al.*, 2006). In some cases, this shift has been associated to an advanced disease profile (Welsh *et al.*, 2005). One early study described the failure to respond to the intradermal injection of tuberculin (skin test and gamma-interferon) in advanced and generalised infections (e.g. ‘anergic’ animals), but a certain proportion can be detected using antibody tests (Yearsley *et al.*, 1998). Therefore antibody response may act as a marker for advanced disease (potentially more infectious cases) or it may have a role in the development of disease by down-regulating cell mediated control mechanisms (Hussain *et al.*, 2001).

Early studies using crude mycobacterial preparations provided satisfactory test sensitivity but resulted in poor specificity due to broad cross-reactivity with non-TB mycobacteria such as *M.avium* (O’Loan *et al.*, 1994; Gaborick *et al.*, 1996). Many attempts have been made to identify immuno-dominant proteins with improved specificity (Amadori *et al.*, 2002; Koo *et al.*, 2005). The most promising antigens used for sero-diagnosis to date appears to be MPB70 and MPB83 (McNair *et al.*, 2001). These appear to be *M. tuberculosis* complex specific and are likely to be the core reagents in any antibody based test for bTB (Wiker, 2009). Recent advances in both antigen discovery and immunoassay technology have facilitated progress in developing novel antibody-based tests for bTB with studies demonstrating the benefit of using multi-antigen approaches

(rely primarily on MPB83 plus additional proteins) to improve the overall sensitivity (Lyashchenko *et al.*, 2008; Whelan *et al.*, 2010b).

Overall, antibody-based tests offer the possibility of convenience, flexibility and more cost effective platforms for bTB surveillance (Schiller *et al.*, 2010a). However, *ante-mortem* tests of cellular immunity can identify *M. bovis* infected animals earlier and have greater sensitivity than the antibody-based assays evaluated to date (de la Rua-Domenech *et al.*, 2006).

1.1.6.2 *Post-mortem*

Due to the lack of clinical and characteristic signs of bovine tuberculosis in most cattle, the diagnosis of the disease by clinical examination is of very limited value (Schiller *et al.*, 2009). Instead direct diagnosis depends upon identification of the organism following *post-mortem* examination, which can be in conjunction with *ante-mortem* immunological tests where positive animals are slaughtered or through passive surveillance as part of routine meat inspection protocols (Schiller *et al.*, 2010a). In practice, there are only two broad approaches to directly diagnose *M. bovis* in cattle. They are based upon the detection of the organism in host animals either through direct culture of bacterium or using molecular methods such as the polymerase chain reaction (PCR) technique to identify the presence of bacteria-specific sequences of DNA (Wilsmore and Taylor, 2008). However, the performance of PCR based tests is said to be unreliable, with one study concluding that an optimised PCR test applied to bovine *post-mortem* tissues resulted in a test sensitivity of 61-65% compared to conventional pathology and culture (Parra *et al.*, 2008). Although it is generally accepted that the PCR technique is not yet able to perform as well as conventional bacterial culture in the detection of *M. bovis* in terms of sensitivity, specificity and reliability, but both type of methods suffer from problems of sampling (i.e. samples with low

levels of organism, or inhibitors which prevent efficient PCR reactions) and cost constraints (i.e. time and financial resources) (Schiller *et al.*, 2010a).

It has also been suggested that the success of direct diagnostic tests largely depends on the presence or absence of visible pathological lesions in the carcase and samples submitted to the laboratory and is closely linked to the stage of *M. bovis* infection (Goodchild and Clifton-Hadley, 2006). One potential drawback in detecting visible lesions is the lack of sensitivity (one estimate of 28.5%) given the normal constraints for meat inspection in abattoirs which limits the detail of *post-mortem* examinations (Anonymous, 2009). This is particularly important if *post-mortem* information is used to assess results from other *ante-mortem* tests. For example it is well recognised that most of the conventional immunological tests (notably the skin test and gamma-interferon) are much more sensitive, though compared to *post-mortem* results at face value, these tests would appear to identify a large number of false positive animals (Hartnack and Torgerson, 2012). In reality, most of these animals will be infected but the *post-mortem* surveillance will have failed to identify infection. In other studies, interestingly, the estimated sensitivity of thorough gross *post-mortems* in known infected herds can be as high as 86% (Norby *et al.*, 2004) and a meta-analysis performed by the Veterinary Laboratories Agency (AHVLA meta-analysis study team) suggest a mean of 69% for the slaughterhouse surveillance sensitivity from all relevant studies in the literature, though in reality, there could be variations between different regions and populations (Downs *et al.*, 2011).

While typical gross pathological lesions can be indicative of infection, they are not definitive (Shitaye *et al.*, 2006). Confirmation of infection status can only be reached on using tests such as bacteriology and/or molecular methods described above. Therefore, although in field conditions, histopathology is frequently used as a confirmatory method to slaughterhouse surveillance, it can

normally only be suggestive as lesions defined as granulomatous can be caused by other bacteria (such as *M. avium* or other environmental mycobacteria) (Shitaye *et al.*, 2009). Nevertheless, abattoir surveillance with lesion detection during commercial slaughter is commonly used as a cost-efficient method of passive surveillance of bTB and often a supplement to live cattle testing.

After confirmation of infection through direct diagnostic testing during *post-mortem*, genotyping of bacterial isolates or PCR products can be used to distinguish isolated *M. bovis* strains on a molecular basis (Smith *et al.*, 2006a). Genotyping technique is increasingly becoming a standard tool for epidemiological disease control and eradication, especially in developed economies. It can provide important insights into the sources of infection and identification of practices or environments which may aid the spread and maintenance of tuberculosis (Schiller *et al.*, 2010a). Importantly, transmission routes between livestock and wildlife maybe identified by strain typing (Skuce *et al.*, 2010). Currently in GB, Spoligotyping (Kamerbeek *et al.*, 1997) and VNTRs (variable number tandem repeats) typing (Frothingham and Meeker-O’Connell, 1998) are common genotyping methods used to distinguish between different *M. bovis* strains, though recently more attention is shifting towards whole genome sequencing (WGS) techniques as the future direction (Biek *et al.*, 2012).

1.1.7 Vaccination

Currently the only available vaccine against bTB is the bacille-Calmette-Guerin (BCG), which is a live attenuated strain of *M. bovis* (Wedlock *et al.*, 2007). Though this has shown variable efficacy in cattle trials, which may be attributable to various factors including vaccine formulation, route of vaccination, and the degree of exposure to environmental mycobacteria (Skinner *et al.*, 2001).

Experimental trials have also been conducted on a number of other vaccines, but none has been shown to offer better protection compared with BCG (OIE, 2009). It has been suggested that in countries with no test-and-slaughter control policy, BCG vaccination may be used to reduce the spread of infection in cattle, however, a vaccine would not guarantee 100% protection (some may still contract the disease) and there is no solid knowledge of long-term reduction in the disease prevalence as well as the safety to humans beings and the social environment (Wedlock *et al.*, 2011; Buddle *et al.*, 2011).

It is also important to recognise that the use of BCG vaccine will compromise immunological tests that rely on the use of tuberculin as the diagnostic antigen (e.g. tuberculin skin tests and gamma-interferon) (Whelan *et al.*, 2010a). Therefore it is difficult to distinguish between BCG-vaccinated animal with TB-infected cattle (true test positive cases). And for this reason, it is currently illegal under EU law to vaccinate cattle with BCG (Defra, 2013b). However, significant progress has been made in the development of so-called DIVA (Differential diagnosis of infected from vaccinated individuals) antigens that allow the differentiation of BCG vaccinated from *M. bovis* infected animals, particularly when used in the gamma-interferon test (Vordermeier *et al.*, 2011a,b). But even when this has been fully developed, it will have limitations in test sensitivity and specificity (Conlan *et al.*, 2015) as well as the need to go through EU and international approval. Nonetheless, BCG vaccination and DIVA test can potentially reduce the progression, severity and excretion of bTB, resulting in reduced transmission between animals (Conlan *et al.*, 2015; Chambers *et al.*, 2014). Cattle vaccination alone will not be sufficient to eradicate bTB in the UK, surveillance programmes must combine other control measures including reducing the spread of *M. bovis* in wildlife reservoirs of infection. Preliminary evidence from limited number of studies has shown that vaccination in wildlife populations can reduce the risk of bTB infection (Carter *et al.*, 2012; Gortazar *et al.*, 2011), although factors such

as the population density, local prevalence, threshold for herd immunity, and the capture rate can all have important implications on the efficacy of vaccination programmes (Byrne *et al.*, 2012; Abdou *et al.*, 2016). Simulation models developed by Abdou *et al.* (2016) predicted that vaccination strategies in wildlife can be effective in reducing bTB prevalence in badgers when combined with culling strategies. Though it was suggested that the benefits of vaccination as a means of reducing bTB in wildlife and subsequently in cattle would take a long period of time (i.e. decades) before being realised (Gormley and Corner, 2011).

1.2 Bovine tuberculosis in GB and Ireland

Great Britain (GB) and Northern Ireland (NI) experienced a very high bTB incidence in 2002, which has been attributed to the suspension of bTB testing during the Foot and Mouth (FMD) epidemic and the widespread restocking post FMD throughout the country with untested / infected cattle (Carrique-Mas *et al.*, 2008). The Republic of Ireland (ROI) escaped both the FMD and its bTB consequences with data indicating a low and stable bTB incidence (Figure 1.1). England and Wales, continues to experience a steady increase in bTB cases after 2002, while Scotland managed to maintain a very low and stable situation of bTB. Immediately after the FMD epidemic, NI quickly implemented enhanced testing and cattle control strategies (Figure 1.2), which has resulted in a 50% reduction in bTB incidence in the region. Furthermore, evidence from Abernethy *et al.* (2013) demonstrates that the differences in bTB incidence between GB and Ireland correlate strongly with cattle testing and disease control measures implemented in the respective jurisdictions, though the massive effort in wildlife intervention strategies in ROI may also be a significant factor (Byrne *et al.*, 2014).

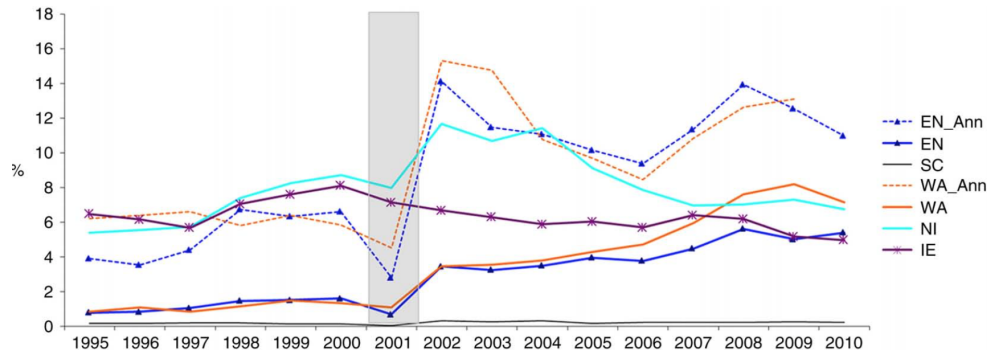


Figure 1.1: Standardised annual herd incidence. EN, England; SC, Scotland; WA, Wales; NI, Northern Ireland; IE, Republic of Ireland; Ann, annually tested regions; shading represents duration of foot-and-mouth disease epidemic. (Figure reproduced from *Bovine tuberculosis trends in the UK and the Republic of Ireland* (Abernethy et al., 2013)).

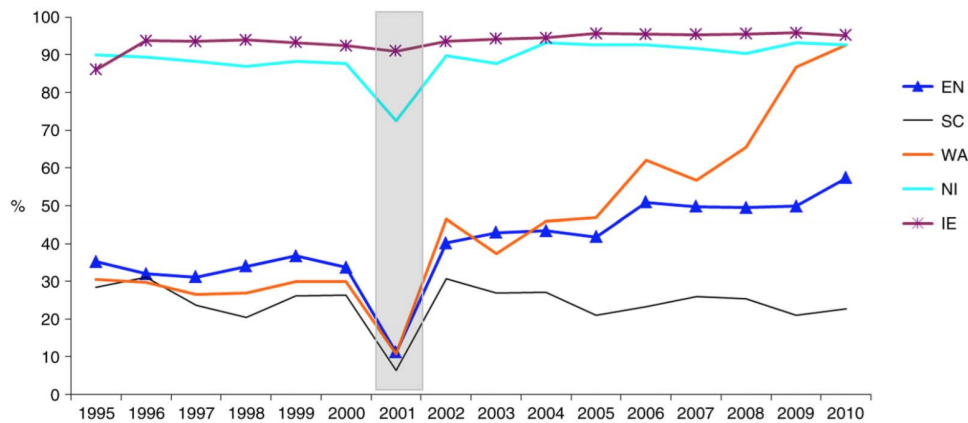


Figure 1.2: Herd test coverage. EN, England; SC, Scotland; WA, Wales; NI, Northern Ireland; IE, Republic of Ireland; shading represents duration of foot-and-mouth disease epidemic. (Figure reproduced from *Bovine tuberculosis trends in the UK and the Republic of Ireland* (Abernethy et al., 2013)).

Recently published bTB statistics shows that there were peaks in bTB incidence during 2008 and 2012 in Wales, following which there has been a decline and stabilisation of the trend. After a peak in England in early 2013 the trend appears to have stabilised (Figure 1.3). However, for both England and Wales it is unknown whether this is a part of a new longer term trend. The most recent figure in March 2016 shows that the total number of animals slaughtered for bTB control (include test reactors, direct contacts and inconclusive reactors) is 28,900 and 8,711 in England and Wales respectively, this represents a 8% and 39% increase compared with 2015, while in Scotland, only 139 cattle were slaughtered during the same period (Defra, 2016). In Northern Ireland, a total of 11,283 animals were slaughtered since April 2015 (Defra, 2016).

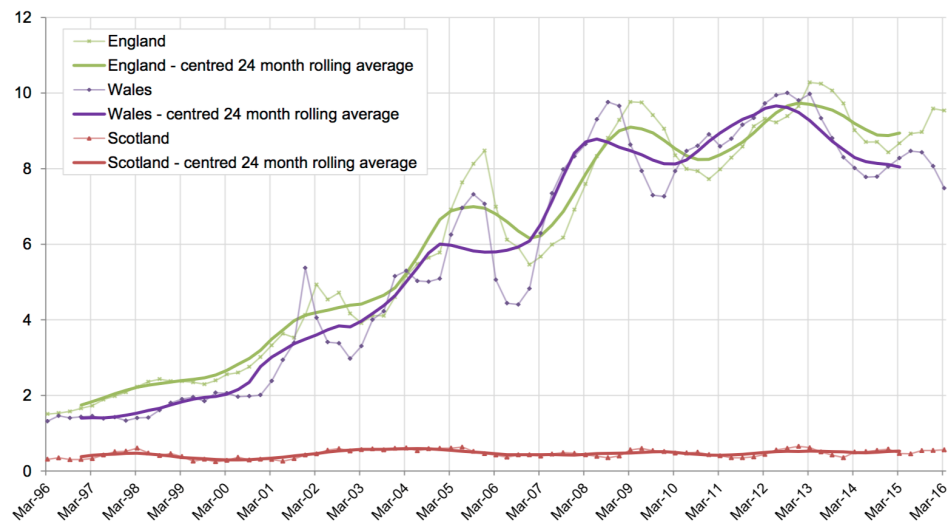


Figure 1.3: New herd incidents (measured in March) per 100 herd years at risk of infection during each year in GB (y-axis). These statistics are obtained from the Animal and Plant Health Agency (APHA) work management IT support system (Sam), used for the administration of bTB testing in GB. They are a snapshot of the position on the date on which the data were extracted. (Figure reproduced from the *Quarterly report on the incidence and prevalence of bovine tuberculosis (bTB) in Cattle in Great Britain by Defra (Defra, 2016)*).

1.3 The bTB testing and control strategy

Historically, the bTB control policy within the UK was determined separately for GB (encompassing England, Scotland and Wales) and Northern Ireland (along with the Republic of Ireland, is located on the island of Ireland). Compulsory national eradication programmes was commenced in the UK and the Republic of Ireland since the mid 1950s (Abernethy *et al.*, 2013). Although following entry of both countries into the EU in 1973, surveillance and control programmes of bTB were largely standardised through the European legislation, principally 64/432 EEC and 78/52 EEC. However, due to different administrations, geography (GB, island of Ireland), epidemiological features and risk factors for bTB, the surveillance strategy and disease management have been developed based on regionalised approaches. In addition, due to the declaration of Officially bTB Free (OTF) status in 2009, Scotland has a separate bTB control policy to the rest of the UK (discussed in details in next section).

The standard control measure applied for bTB in UK cattle herds is test and slaughter; with the current intensive surveillance programme consisting of routine on-farm testing of cattle and subsequent removal (slaughter) of test positive and in-contact animals. All reactors that were slaughtered undergoes *post-mortem* meat inspection. In addition, samples were taken from a number of animals during *post-mortem* inspection and were sent to the laboratory for *M. bovis* culture. If a reactor is identified, the herd is classified as having a breakdown, and is subject to further testing with other control measures including cattle movement restrictions. If *M. bovis* infection is confirmed through the observation of macroscopic lesions typical of bTB in one or more cattle during *post-mortem*, or positive histopathology or culture of *M. bovis* from tissue sample is made, the breakdown is then described as ‘confirmed-breakdown’. Typically, the start

date of a bTB breakdown is the initial disclosure of a reactor or infected animal (e.g. detected through regular slaughterhouse surveillance), and the Officially Tuberculosis Free (OTF) status is suspended or withdrawn. The end of the breakdown is the date following effective control measures, and when movement restrictions were eventually lifted after two consecutive clear whole-herd tests using SICCT at minimum intervals of 60 days apart. A six-month post-outbreak test usually takes place following reinstatement of the OTF status. A detailed process that triggers a change in the OTF status is shown schematically in Figure 1.4. In GB, should infection not be confirmed, the OTF status is suspended and only one further negative herd test is required. Northern Ireland has a similar policy, except that outbreaks with more than five unconfirmed reactors are treated as OTFW (Officially bTB free withdraw), while almost all outbreaks in the Republic of Ireland are considered as OTFW (Good *et al.*, 2011).

The primary *ante-mortem* diagnostic test used to establish infection during routine surveillance is the SICCT test with avian (2500 IU per dose) and bovine (3000 IU per dose) tuberculins manufactured by Lelystad (Abernethy *et al.*, 2013). Under the EU legislation, the frequency of routine surveillance is determined by the prevalence of infected herds (Anonymous, 1964). In Scotland, the only officially bTB free parts of the UK, 4-year routine herd testing has been ongoing for at least 15 years. Though recent changes were introduced to exempt low risk herds from the default test interval and the exemption eligibility is reviewed annually. By contrast, annual herd testing has been carried out in the Republic of Ireland and Northern Ireland for many years (Byrne *et al.*, 2015).

Historically, the frequency of routine herd testing in England and Wales have been calculated by monitoring the level of bTB in a given area in the previous six years (Defra, 2013a). The test interval ranges from 1 (a whole herd test (WHT)) to 2-4 years (a routine herd test (RHT)), depending upon the history of bTB

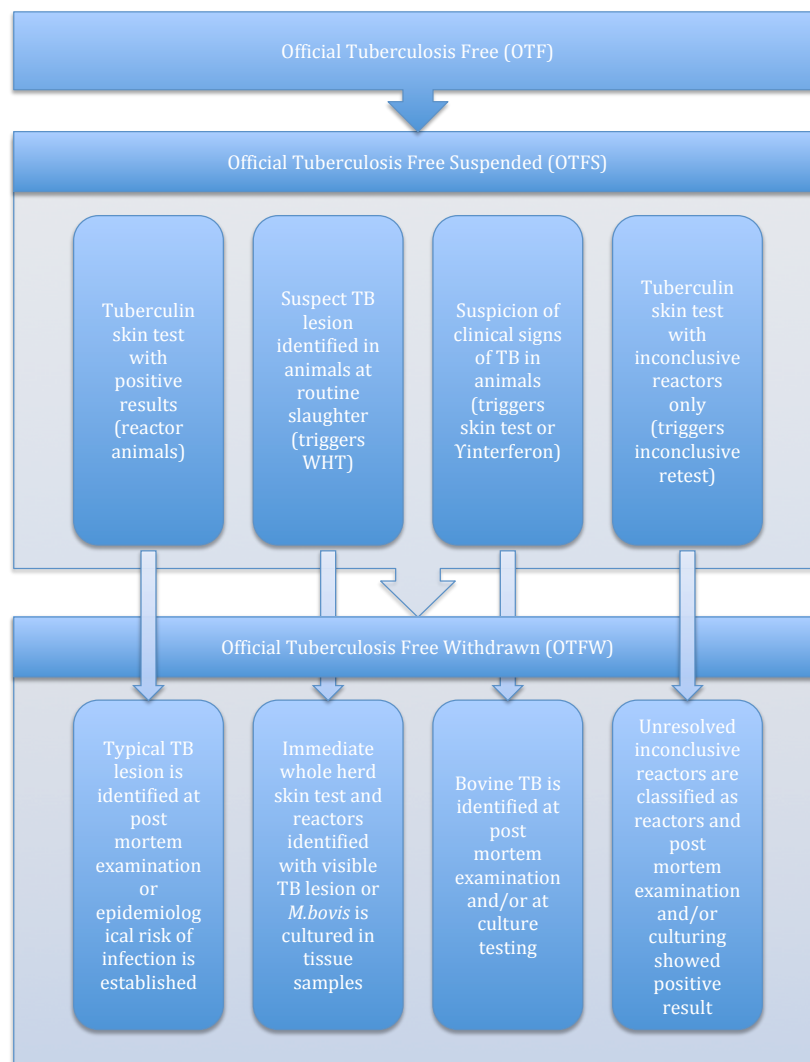


Figure 1.4: Events or processes that triggers the suspension or the withdrawn of an Officially Tuberculosis Free (OTF) status in a otherwise OTF herd

in the herd and its surrounding geographical areas (Green and Cornell, 2005). Where bTB is thought to be more prevalent, the testing interval is shortest (1-year) and where the threat of disease is considered minimal, herds are tested at 4-year intervals. The calculation of bTB testing intervals based on historical incidence was seen as ‘reactive’ approach, and in some areas of expanding or emerging bTB incidence, this trailed behind the spread of infection (AHVLA, 2012). Additionally, parishes are not the most suitable geographical unit on which to assess herd incidences because of their small size, and their often awkward shape (AHVLA, 2012). In some cases, this meant that annual and four-yearly parishes could be found next to each other. Since 1 January 2013, a new and more proactive bTB testing regime with a more risk based approach was introduced. The bTB testing intervals for bovines was determined on a county basis rather than by parishes (pre 2013) and were tested either annually (6-monthly test were used for herds in edge area part of Cheshire) or four-yearly (Brooks-Pollock *et al.*, 2014), resulting in more stable routine testing frequencies. This approach was more proactive than the previous arrangement, because it aimed to get ahead of the advancing front of infection in high bTB incidence and risk areas (AHVLA, 2012). By setting the bTB testing intervals on the basis of the current disease picture, a more coherent distribution of testings across the country was adopted, which is more consistent with the risk and the epidemiology of bTB in each region. See Figure 1.5 for the most recent testing frequency in GB.

The slaughterhouse surveillance is undertaken for all cattle by meat inspector. The inspection protocols are standardised through European legislation, with samples of macroscopic lesions submitted for laboratory confirmation or from a pool of lymph nodes if no lesions are detected. Where bTB is confirmed in cattle slaughtered as part of routine farm production (so-called “slaughterhouse cases”), the herd follows the same restriction and test regimen as one with confirmed bTB reactors.

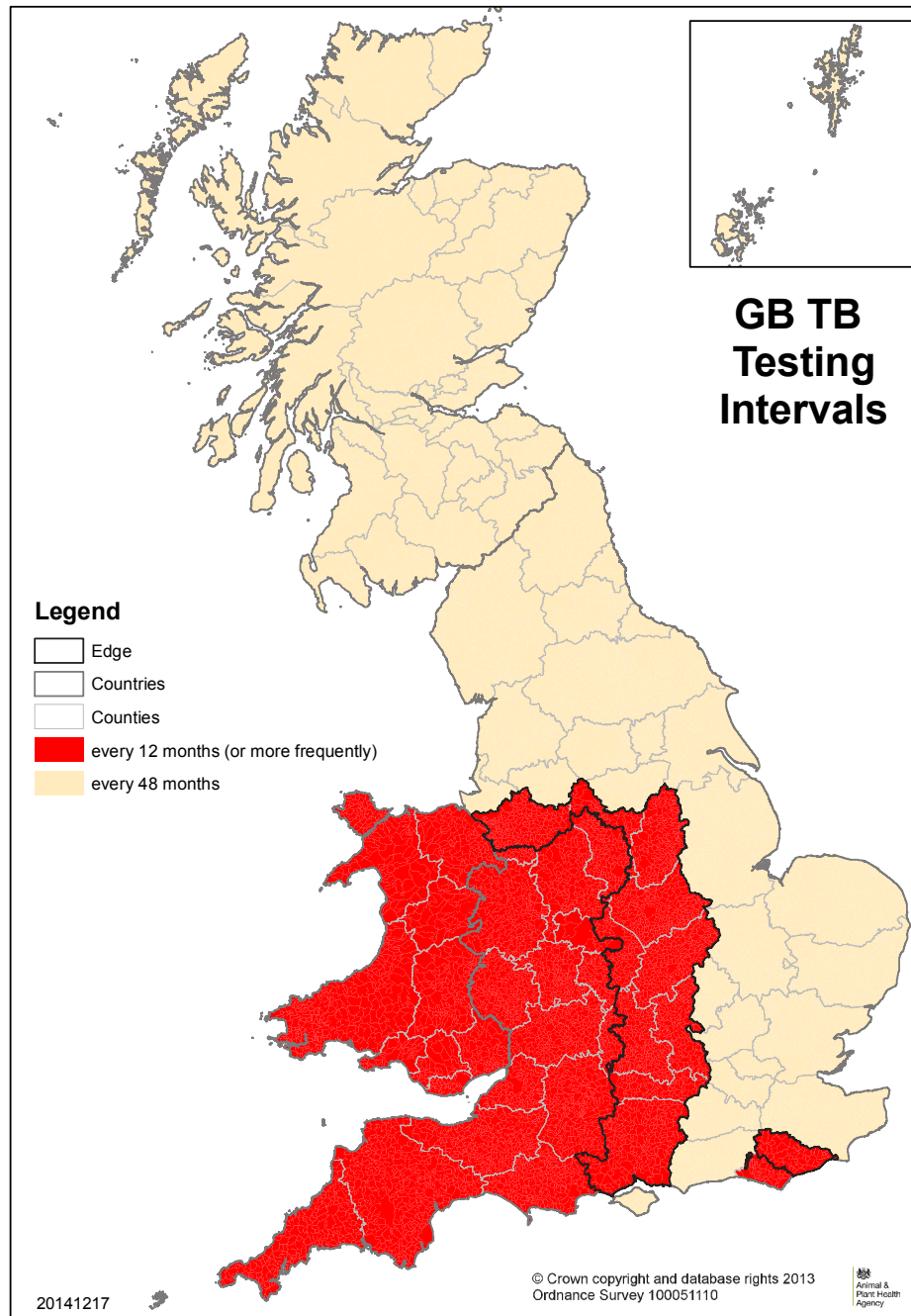


Figure 1.5: Map of Great Britain showing the bTB testing intervals for 2016 published by Defra

1.3.1 Movement testing and additional control measures

Recognising the importance of cattle movements in spreading bTB, compulsory pre and post-movement testing was gradually introduced between 2005 - 2016 in England, Wales and Scotland (Anonymous, 2016b). The main objective of the test is to protect healthy herds that are importing cattle from endemic areas by quickly identify and remove infection before disease could spread to other animals or herds. The legislations requires all cattle (42 days old or over) imported from high incidence areas (annual or more frequent surveillance testing areas) to test negative for bTB in a SICCT test within 60 days before movement and between 60 - 120 days post-movement at the receiving farmer's expense (Gates and Volkova, 2012). Surveys have estimated the direct veterinary costs of testing range from £5.50 to £9.00 per animal, although the actual costs may be higher due to labour expenses, disruptions in farm business practices, and missed marketing opportunities (Bennett, 2009). While a government funded surveillance test scheduled to take place 60 days prior to move or within 60 - 120 days post-movement testing window may also be considered valid pre- or post-movement tests.

As well as tests associated with routine surveillance and movement testing, other additional type of control measures may be applied depending on circumstances under consideration. Such as contact tracing to herd of origin and neighbourhood test (infected and surrounding herd or contiguous herd test within 3km radius) after declaration of a herd breakdown (Defra, 2014b). Gamma-interferon blood assay may also be used in conjunction with the SICCT test in higher risk breakdown herds to increase the diagnostic sensitivity and identify potential early stage infections.

1.3.2 The bTB control in Scotland

Scotland records very few incidences of bTB in recent years (149 animals were slaughtered for bTB control in 2016, these include reactors, inconclusive reactors and direct contacts) (Anonymous, 2016a). And majority of these cases can be traced back to imports from endemic areas of England, Wales or from Republic and Northern Ireland (Gates *et al.*, 2013). As a result, Scotland has been designated as an OTF region in September 2009 for the purposes of cattle trading. Under the provisions of Council Directive 64/432/EEC, OTF status does not imply that *M. bovis* is absent from the domestic herd, but is instead awarded to a territory where both the average annual incidence and prevalence of bTB amongst cattle herds has remained below 0.1% for six consecutive years and appropriate surveillance programmes are in place to detect new herd breakdowns (Anonymous, 2009). Although Scotland has successfully maintained bTB incidence below 0.1%, new breakdowns continue to be identified through routine surveillance each year (Gates and Volkova, 2012). Due to Scotland not being a fully OTF member state (non-OTF in England and Wales), there is a significant and continuous risk of disease incursion from neighbouring countries (i.e. import of infected cattle from bTB endemic regions).

The surveillance and control strategy for bTB in Scotland consists of a combination of pre- and post- movement testing (SICCT tests) of cattle (60 days before and after movement) and implementation of a risk-based herd testing strategy, with eligible herds on a four-year routine herd testing (RHT) cycle, which identifies approximately 1/3 of the incidences of bTB, but accounts for the majority of active screening that takes place (Scottish-Government, 2011). Further inspections of carcasses for evidence of bTB lesions at slaughterhouse detect additional 1/3 cases of infection. The remainder are detected through other forms of surveillance including epidemiological tracings and additional tests.

Recent changes in risk-based routine surveillance testing introduced exemptions for low risk herds from the default routine testing interval of 4-years which applies to all other non-exempt herds (Scottish-Government, 2011). The eligibility for exemption from bTB testing is reviewed and assessed annually by the APHA. Low risk herds must fully comply with one of the following:

- Herds with fewer than 20 cattle which have had fewer than 2 consignments of cattle moved on from high incidence bTB areas (including Northern Ireland and the Republic of Ireland) in the previous 4 years.
- Herds that slaughter more than 25% of their stock annually and have had fewer than 2 consignments of cattle moved on from high incidence bTB areas (including Northern Ireland and the Republic of Ireland) in the previous 4 years.
- Herds that slaughter more than 40% of their stock annually.

The slaughter rate is calculated on the total number of cattle slaughtered in a slaughterhouse in the previous calendar year divided by the herd size (total stock on farm on 1 January).

1.4 Cattle movement and bTB databases

1.4.1 Cattle Tracing System database

The first requirement to identify individual cattle was introduced in Great Britain in 1953 as part of national efforts to eradicate bovine tuberculosis. This legislation was extended in 1960 with the Movement of Animal (Records) order,

which require farmers to keep a record of all movements of animals on or off their premises for at least 3 years. In the 1990s, in response to the growing concerns over BSE (Bovine spongiform encephalopathy), the Bovine Animals (Identification, Marking, and Breeding Records) Order was introduced to ensure that the birth of calves and the identity of their dams were recorded within 36 hours of birth for dairy cattle and 7 days after birth for all other cattle. To comply with Council Directive 92/102/EEC issued by the European Economic Community in 1992, the Bovine Animals (Records, Identification and Movement) Order was introduced in 1995. The legislation required all farmers to register their holding with the local Animal Health Office and all cattle to be issued a unique ear-tag number (consisting of no more than 14 characters). Subsequently, following the BSE crisis, a cattle passport scheme was introduced in July 1996, where all farmers were required to register the birth date, sex, breed and parenting of newborn calves on the farm so that a physical passport could be issued by the local agricultural authority. This scheme was later incorporated into the British Cattle Movement Service (BCMS) in 1998, and subsequently led to the establishment of the electronic Cattle Tracing System (CTS) database to manage the large volume of cattle records. Since January 2001 it has been mandatory for livestock-keepers to notify the BCMS of all cattle births, deaths and movements for recording on the CTS data archive. This greatly improved the quality of movement records stored in the CTS database and contribute towards disease control activities (Mitchell *et al.*, 2005; Green and Kao, 2007). The CTS database constitute part of the core information source for the Rapid Analysis and Detection of Animal-related Risks (RADAR) project, run by the Department of Environment, Food, and Rural Affairs (Defra).

Farm registration

All agricultural holdings in the UK that house cattle, sheep, goats, and pigs

must be issued a unique County Parish Holding (CPH) number to report livestock movements and to apply for agricultural subsidy payments. The number consists of 2 digits county code, 3 digits parish code, and 4 digits holding number in the following format: CC / PPP / HHHH. The holdings are classified into different types including farms, livestock markets, calf collection centres, dealer, veterinary practices, slaughterhouses, common grazing land, and other holding facilities. The current regulations for cattle keepers allow all fields and buildings within a 10 mile radius of the main farm site to be registered under a single CPH number. However, farms that manage cattle on multiple uniquely identified land parcels can apply for a ‘linked holding’ status in the CTS, in order to reduce the burden of movement reporting. Linked premises may include grazing land that fall outside of the 10 mile radius or farms that share facilities such as milking parlours. Cattle movements are still required to be recorded between linked holdings in the farm register, but does not need to be reported centrally to BCMS. This may cause discrepancies in the animal’s life history, for example, if an animal was born on the main farm location, then moved onto a linked premise for fattening, and later moved off the linked holding to slaughter.

For landless keepers who raise livestock on rented land or farmers who wish to register seasonal grazing pastures separately from the main holding for subsidisation purposes, a temporary CPH number (identified by a holding number between 7000 - 7999) may be issued. Over time, the CPH numbers assigned to individual locations may change through the conversion of land to cattle farming or other agricultural purposes, new ownership transfers, or the creation of single holding by merging multiple land parcels.

Movement recording

Every newborn calf in the UK is required to have an ear-tag fitted in both ears after birth, and farmers must apply for a cattle passport within 7 days

of tagging. The passport contains information on the animal's ear-tag number (consists of the country code, herd mark, and individual animal number), breed, sex, genetic dam, and date of birth. Cattle that are imported to the UK must also be issued a passport unless they are to be slaughtered within 15 days. Stillborn calves or calves that die before being tagged are not required to be reported, however, records of these animals must be kept on farm. Animal (tagged) death on farm must be reported and the passport returned to BCMS within 7 days.

A cattle movement is defined as the movement of a live animal 'on' or 'off' a holding. This could be initiated in the form of private sale between farms or trading through markets (even if the animal is not sold and subsequently returns to original farm), slaughterhouses, showgrounds, and separately managed, but unlinked holdings. Regulations requires the movement of all cattle to be reported within 3 days of occurrence from both the sender and receiver to reduce potential errors in the database entry. In addition, farms may be periodically inspected and audited to ensure that animal identification and record-keeping were kept accurate.

Database structure

There are seven primary data tables in the CTS database. They provide detailed information on the movements and demographic characteristics of individual animals and livestock locations. A schematic representation of the database structure is shown in Figure 1.6. The CTS data extract used in this thesis for analysis contained all records through to December 2011.

The three tables on the far left in Figure 1.6 contains information on locations where animals may be held. The CTS location data table describes the location type (such as agricultural holding, landless keeper, market, showground, slaughterhouse, and various other location types), location address (such as

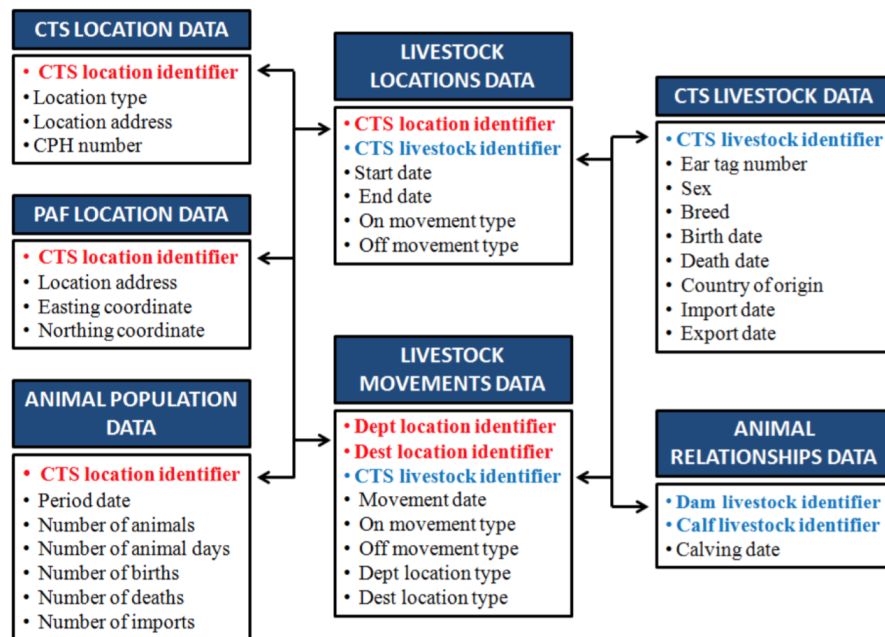


Figure 1.6: Schematic representation of the Cattle Tracing System database structure and relationships between data tables (Source: (Gates, 2013)). Arrows indicate primary identification keys (highlighted in red and blue) used for linkage between tables.

business name, street, town, county and postcode), and the unique CPH number. The location details for some larger farm business may be recorded using the main farm address rather than the actual location where cattle are kept. Some slaughterhouses uses the official 4-digit EEC abattoir code instead of the standard CPH format. This table can be used to link farm data with the CTS movement records to identify information on each residential farms or other national animal health databases and survey studies. The Postal Address File (PAF) table describes the easting and northing coordinate, which can be used for georeferencing each farm locations. However, this information is only available for approximately 65% of locations listed in the CTS database (Mitchell *et al.*, 2005). The Animal population table provides summary statistics associated with each location in a given calendar month, including information such as the total number of animals (cattle, sheep, goat and pigs, if applicatble), animal days, number of births, deaths and import movements. This table can provide information on the average herd size in a given time period, and to potentially identify the nature of the herd (whether it is breeding or fattening herds).

The two data tables in the middle of Figure 1.6 describes the movement and location history of individual cattle. They are derived from the unpaired ‘on’ and ‘off’ movement records submitted to BCMS by cattle keepers. The Livestock locations table is arranged with location as the primary field, along with information on the identity of the animal and the nature and duration of the stay (date of arrival and departure, types of arrival and departure such as birth, movement, or death) on the specific location. This information can be used to trace cattle that were present on any given location on any given date. The Livestock movements table provides paired on and off movements used to identify cattle transfers between livestock locations. Each observation contains information on the animal’s identity with the associated paired location details (such as departure and destination id, location type and movement date) along

with the type of movement (birth, movement or death). Data in this table can be used to construct cattle movement networks and when linked with the PAF table can provide useful data for spatial analysis.

The final two data tables on the far right describes detailed demographic information recorded in the animal passport. The Livestock data table contains animal's ear-tag number, sex, breed, birth date, death date (if applicable), country of origin (if imported from overseas), and date of import and export (if applicable). Animals that were entered into the CTS database retrospectively are frequently missing a birth date or a mandatory date of '2001-01-01' was often used for administration purpose. The Livestock relationships table identifies link between each calf and dam via their identification number along with information on each calving events such as date and sire's identification number (if applicable). This table can be used to generate a list of calving events for each dam and also can be linked with the movement and location data tables to deduce the animal's production purpose.

Data limitations

The method of data recording under the CTS database are gradually moving onto an electronic based system. However, all extracts used for analyses in this thesis were based on the traditional method of data collection where data were obtained using a range of mechanisms including written records submitted by the farmers through post, telephone survey and dedicated website. As with any data source, the CTS data are subject to errors and omissions (Mitchell *et al.*, 2005). If the errors are random, any conclusions based on the data will usually be robust, although the errors will reduce their precision. However, any systematic bias may compromise the utility of the data more seriously. So it is important to identify the distribution of errors where movements are consistently unreported or reported incorrectly. In addition, when missing information are inferred automatically via

best guess, this could cause problem if large proportion of records are estimates rather than actual. These limitations combined with the time delays in reporting livestock movements limits the ability for the CTS records to monitor spatial and temporal trends in performance that may serve as early indicators of disease incursion (Carpenter, 2001; Perrin *et al.*, 2012).

Currently, the CTS database holds more than 170GB (gigabytes) of data on more than 20 million cattle in Great Britain, and the livestock identification and tracking system costs government and the livestock industry around £55 million a year - just over £2 an animal (Bourn, 2003).

1.4.2 The Sam's IT system

Monthly bTB statistics have been published by Defra since 1996. This was the first year that administration of bTB testing was computerised, with records held on the Animal Health and veterinary laboratories agency (AHVLA) old VETNET computer system. In September 2011, AHVLA updated this database and rolled out the TB module of its new computer system, named "Sam". Since October 2014, AHVLA was merged with parts of the Food and Environment Research Agency (Fera) to form the Animal and Plant Health Agency (APHA), which is now responsible for the management of Sam. Sam's IT system contains results of all *ante-mortem* bTB tests in GB and information on suspected and confirmed cases identified through slaughter surveillance along with *post-mortem* diagnostic results if available. There were 3 separate tables in the Sam's IT system: Animals table, Breakdown incidence table and Herd test table.

When a positive (from SICCT or gamma-interferon test) or inconclusive reactor is identified, the passport number of the animal is recorded in the Animal

table along with the test type and any follow-up test results or actions taken (e.g. re-test or slaughter). Although *post-mortem* diagnostic results (identification of visible lesion or positive cultures of *M. bovis* from tissue samples) were also recorded (if available), for herds with multiple reactors, only one positive *post-mortem* case is required to confirm infection. Therefore not all test positive animals were cultured or confirmed of infection through *post-mortem* examination. In addition, there were also histopathology and genotype results from a small proportion of test positive animals with suspect lesion(s) detected at slaughter, however, these data are limited.

The identification of a test positive animal (either from bTB test or *post-mortem* at slaughter) triggers a herd breakdown, subsequently a separate breakdown incident record is created in the Breakdown table, which contains aggregated information at herd-level including: the start and end date of the breakdown incident, total number of test positive animals and the number of animals slaughtered as a result of suspected bTB infection.

Whilst positive bTB tests were reported at animal-level, negative test results are only recorded on a herd-level basis with the following summary information in Herd test table: number of cattle tested, total number of animals in the herd, date and type of test, herd production type, and administrative information for the farm including the county-parish-holding (CPH) identifier of the main farm business, the farm address, and the farm coordinates.

The Sam's IT system are linked with the CTS database via the animal's ear-tag number (CTS Livestock data table in Figure 1.6) and the location CPH number (CTS location data table in Figure 1.6).

1.5 Conclusion

Bovine tuberculosis is a major livestock disease in the UK, and can have significant impact on the sustainability of the livestock industry, both socially and economically. Due to the chronic and silent spreading nature, combined with persistent wildlife infection as well as imperfect diagnostic tests available, the disease is difficult to detect and hard to eradicate. Although there have been tremendous advances in our understanding of how the disease progress and the performance of the diagnostic tests, more research is needed. Detailed information on industry demographics and livestock movement recorded in national cattle movement database (CTS) and the bTB testing data provides a valuable opportunity to conduct these analyses.

1.6 Thesis objectives

This thesis is structured as a series of analyses that illustrates why bTB is difficult to control in the current epidemiological environment, how insights from simulation models with empirical data can be used to guide the development of more effective routine surveillance programmes, and why there is a need for further research into the underlying factors that can depress the immune response to the diagnostic tests and potentially contributes to missed infection.

Chapter 2 provides an empirical analysis to estimate the within-herd incidence rate of bTB for each confirmed breakdown herds in Scotland. Using reactor information from the Sam's IT system combined with the CTS movement database, the study aims to identify the most likely source of disease introduction,

and based on detection of subsequent reactors (secondary infection) for the duration of disease exposure, an average rate of within-herd incidence is calculated. The results indicate that there is considerable variation in the within-herd incidence rate between farms, moreover, herd size and duration of disease exposure are significant risk factors associated with high rate of within-herd incidence.

Chapter 3 explore several different disease scenarios of bTB spread while using a number of alternative routine surveillance test as intervention strategy. Stochastic simulation models were used to simulate bTB spread within a theoretical contact network of farms while routine surveillance testing were applied at a pre-determined time point with variable intensity and frequency. A few key epidemiological parameters for bTB were also investigated to examine the potential impact on the disease spread in the network. Results have shown that with increased surveillance effort (i.e. more frequent routine surveillance activities), low level of internal and external force of infection, and improved diagnostic test sensitivity all lead to substantial reduction in bTB incidence.

Chapter 4 and 5 contain a series of case-control studies to investigate stress-related factors that can potentially suppress the immune response to commonly used diagnostic test (namely, the SICCT test and the gamma-interferon blood assay), and illustrate some key challenges to identify bTB infection. Chapter 4 uses bTB testing data (Sam's IT system) in high-risk areas of the UK to demonstrate that recent calving event is significant factor that can impact the response, directly related to the outcome (positive or negative), for the standard SICCT test and gamma-interferon blood assay. When reactors were matched with non-reactors based on age, breed, and farm of origin, recent calving event (parturition within 60 days of test) was shown to be significant factor that negatively associated with been identified as reactor, and this effect is more substantial the closer the parturition is from the administration of the test.

In Chapter 5, a different case-control study design was used, where SICCT test negative animals that were later confirmed with infection through *post-mortem* diagnostics (i.e. false negative outcome from SICCT) were compared with confirmed SICCT test positives (true positive outcome from SICCT). An expanded list of ‘stress-related’ factors were examined, including recent movement, recent testing as well as recent calving. Results suggest that conducting SICCT test closer to animal movement date or previous SICCT test, can lead to increased odds of false negative outcome.

Chapter 6 summarises all the findings and finishes with a critical discussion of the study limitations and how the results and modelling approaches from this thesis can be used to support the development of more sophisticated epidemiological models in the future.

Chapter 2

Empirical estimation of within-herd incidence rates of bovine tuberculosis in cattle herds in Scotland

2.1 Introduction

Bovine tuberculosis (bTB) is a chronic disease of animals caused by infection with the slow-growing, obligate intracellular bacterium *Mycobacterium bovis* (Bourne, 2007; OIE, 2009). Pathogenesis studies reveal that bTB is predominately a respiratory disease and the majority of infections are thought to occur via ‘direct’ aerosol transmission between animals in close proximity (Menzies and Neill, 2000). Onward transmission appears to require lesions in the lungs and associated lymph nodes, although many are too small to be detected routinely at

abattoir meat inspection (McIlroy *et al.*, 1986a). Domestic cattle is the preferred host of *M. bovis*, though wild animal populations such as badgers, possums and cervids may also become maintenance hosts (Krebs, 1997; Porphyre *et al.*, 2008; Munroe *et al.*, 1999). Most other animals act only as spillover hosts (i.e. become infected but do not usually transmit the disease) (Neill *et al.*, 2005). In countries with advanced test and control programmes (a comprehensive set of surveillance and control measures to address disease transmission) bTB is a low incidence infectious disease with an apparently low transmission rate (Munroe *et al.*, 2000). Infection would appear to be relatively poorly transmitted between cattle in most, but not all, circumstances (Skuce *et al.*, 2011). Though several recent studies demonstrated clear evidence of ongoing cattle-to-cattle transmission within herd, after introduction of one or several infected cases (Bourne, 2007; Perezill *et al.*, 2011; Alvarez *et al.*, 2012; Gates *et al.*, 2013).

Important factors that contribute to cattle-to-cattle transmission include the frequency of bacteria shedding, infective dose, disease exposure time, level of cattle-cattle interaction and host susceptibility (Goodchild and Clifton-Hadley, 2001). In addition, the within-herd dynamics of bTB are further complicated by the disease's long incubation period (Alvarez *et al.*, 2012). The chronic nature of infection means that it can take from weeks to years to develop from infection to clinical disease and to become infectious to other animals (OIE, 2009), this gives a large time frame for the disease to potentially spread undetected within the herd. However, the time of infection and the conditions under which a tuberculous animal becomes an effective disseminator of infection are not well defined and difficult to estimate (Menzies and Neill, 2000). Furthermore, the infectiousness appears to vary with time post-infection (Goodchild and Clifton-Hadley, 2001). This is thought to be related to a late stage test unresponsive period, most often characterised as a state of anergy (Barry *et al.*, 2009; Young *et al.*, 2009), where

animals are in advanced stages of infection and is no longer responsive to cell-mediated immunological tests (tuberculin and gamma-interferon tests).

Little is known as to which animals transmit the most, although current epidemiological evidence and modelling studies are lending support to the concept of the ‘super-shedder’ (or super-spreader) animals to account for heterogeneity in the infectiousness of individuals (Gardy *et al.*, 2011; Skuce *et al.*, 2011; O’Hare *et al.*, 2014), but this phenomenon remains largely unexplained. Experimentally, an animal may become a ‘super-shedder’ if it has high shedding frequency and excretes more mycobacteria than others (Kao *et al.*, 2007). In field conditions however, it is more difficult to determine individual infectiousness, though studies have suggested that among bTB-confirmed cattle, evidence of pathological lesions or successful mycobacteria culture is synonymous with infectiousness and is directly associated to onward transmission (McIlroy *et al.*, 1986b; Menzies and Neill, 2000; Liebana *et al.*, 2008). As a consequence, the average infectiousness of individuals may vary between herds (Gopal *et al.*, 2006) and the efficiency of testing depends not only on the characteristics of the diagnostic test, but also on the competing timescales of transmission (Conlan *et al.*, 2012).

Studies using within herd dynamic models of bTB have been developed to address these issues and indicate a wide spectrum of within-herd spread (Barlow *et al.*, 1997; Munroe *et al.*, 2000; Perez *et al.*, 2002; Alvarez *et al.*, 2012), but to date there is very little information in the published literature about the incidence rate of *M. bovis* infection within infected cattle herds. This chapter presents an empirical analysis using field data to estimate the incidence rate following initial disease introduction in Scottish cattle herds. Whilst the number of bTB reactors in a herd can be observed, the period of disease exposure is crucial in estimating within-herd incidence rate; this is the length between the initial disease introduction and the eventual removal of infection from the

herd. The disease removals can be inferred through back tracing from data collected in bTB testing histories and cross referencing with cattle movement data, however, the exact point of disease introduction are extremely difficult to determine. Evidence has shown that cattle movements are a significant predictor of the introduction of bTB (Gilbert *et al.*, 2005) and poses a clear transmission risk (Gopal *et al.*, 2006), especially to officially bTB free (OTF) herds. With little evidence of an established wildlife reservoir as a transmission route, imports of undetected infected cattle are considered to be the primary method of disease introduction amongst Scottish cattle herds (Gates *et al.*, 2013). The time of disease introduction can therefore be inferred from their corresponding import date once an infectious animal is identified. Reviews on cattle-cattle transmission pathways by Skuce *et al.* (2011) also suggest that new breakdowns occurring in geographically separated, and previously bTB-free regions in Scotland, could be linked to the movement of cattle from bTB hotspot areas as a result of trading. When a clear point of disease introduction is identified, the herd-level incidence rate can be calculated based on the number of secondary infected cases (after initial disease introduction) over the duration of disease exposure from the population of susceptible cattle at risk in the herd. The within-herd incidence rate is examined here in this chapter.

2.2 Materials and Methods

2.2.1 Model structure

The average daily within-herd incidence rate of bTB following an initial disease introduction (in the form of a single infected animal) is calculated using

the simple rate of spread formula as follows:

$$\rho = \frac{I - 1}{N \times d}. \quad (2.1)$$

Where ρ is the daily within-herd incidence rate of bTB, I is the aggregated number of test positive cases within the herd, N is the total susceptible population (i.e. herd size, including infected animals) and d is total number of animal days at risk (also equivalent to the length of disease exposure period).

Specifically, the number of test positive cases consisted of SICCT and/or gamma-interferon reactors with the addition of re-confirmed inconclusive reactors (after subsequent re-test) for the duration of the disease episode (i.e. from initial declaration of infection until confirmation of eventual disease freedom). While the herd population size was averaged over the entire disease episode period based on mean daily count.

The total number of animal days at risk is calculated from the initial date of disease introduction until the date of removal of last detected case(s) before the movement restriction was lifted following two consecutive clear whole herd tests. While it is fairly easy to deduce the removal date of the last reactor (indication of disease freedom), the point of disease introduction is an unknown quantity that needs to be estimated. This is inferred through back tracing of animal movements between two previous points of clear whole herd test and identifying reactors imported (from high risk areas) during this time that can potentially act as an initial disease dissemination source (Figure 2.1).

It is assumed that animal movements are the only route of disease introduction, this may for example be an animal import from high risk areas of Ireland, Northern Ireland, England and Wales, or from a livestock market and farm premises within Scotland and areas with low bTB incidence. Movements

of animals between herds within Scotland are not subject to the pre- and post-movement test, undetected infection could therefore contribute towards the silent spread following the introduction onto disease free herds.

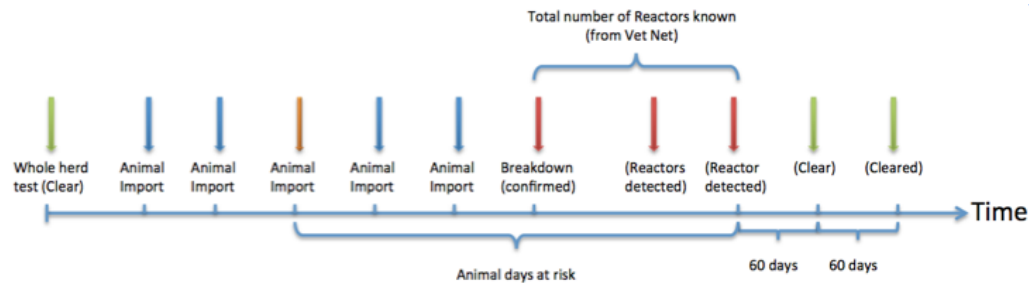


Figure 2.1: Time line of events between two clear whole herd test in a disclosed breakdown herd. The golden arrow in the figure indicate an effective introduction of a potentially undetected ‘reactor’ following the most recent clear whole herd test prior to a breakdown incident. The red arrows represent events following an initial detection, where more reactors were identified through subsequent follow up tests. Green arrows indicate the time of clear whole herd tests, either as a result of routine surveillance or regulatory check tests following a reported breakdown incidence.

The movement date of all reactors (SICCT + gamma-interferon test) and inconclusive reactors in identified breakdown herds were traced. The initial introduction of the infectious source was inferred amongst cattle imports from the period between the previous clear herd test and the date that the breakdown was declared (i.e. between the first green arrow to the first red arrow in Figure 2.1). The exact time of disease introduction is then determined as the movement date of the earliest reactor import during those times. In addition, reactors with positive *post-mortem* diagnostics, such as the presence of TB-like lesions or culturing of *Mycobacterium bovis* were deemed to be the true infectious source in favour of reactors with negative or no *post-mortem* diagnostic results.

2.2.1.1 Assumptions

This methodology has several assumptions:

- High-risk animal imports are assumed to be the only disease introduction route into otherwise OTF herds in Scotland (or other low incidence regions). Thus, absence of evidence of wildlife reservoir.
- Any breakdown incident is initially seeded by a single individual infected animal that is detected on the same farm at a later date and is recorded in the bTB testing database.
- The analysis only consider breakdowns where there has not been a previous disease history in the herd in order to minimise the impact of possible infections missed from previous outbreaks.
- Every cattle herd in Scotland is tested at least once every 4 years. Although recent changes introduced exemption criteria for some low risk herds from routine surveillance (approx. 25%)
- The outcome of two clear RHTs are reliable and accurate indication of disease freedom (i.e. probability of hidden and undetected infection persist in herd after two consecutive clear herd tests is ignored).
- The earliest reactor import was assumed as the initial disease introduction rather than a secondary infection from other reactor imports.
- Reactors with positive *post-mortem* results are more likely to be true reactors rather than false positives and therefore have higher probability of being the initial infectious seed.
- All infected animals during a breakdown episode are detected.

2.2.2 Data

2.2.2.1 Data sources

Cattle test data from the national database of bTB testing history (Sam's IT system) were used, which contains information on each bTB breakdowns reported to Animal Health and Veterinary Laboratories Agency (AHVLA). Details of animal movements and distribution were obtained from the cattle tracing system (CTS) database provided by the Department for Environment, Food and Rural Affairs (Defra). The analysis is based on data from new confirmed herd breakdowns in Scotland between 2002 and 2011 where at least two reactors (including slaughterhouse cases and inconclusive reactors) were detected and slaughtered. This is based on the assumption that disease spread can only be attributable to secondary infection cases from an initial disease introduction source (i.e. undetected reactor import / movement), hence herds with only one reactor animal constitute zero rate of spread and is not of interest to this study. Herd test records after 2002 were used because during the 2001 foot and mouth (FMD) disease epidemic, all bTB testing were suspended for most of the year and overdue tests were carried out gradually after the FMD outbreak (Carrique-Mas *et al.*, 2008).

2.2.2.2 Data processing

Infected cases

The total number of diseased cases in each breakdown herd comprised the number of slaughtered reactors, inconclusive reactors and slaughterhouse cases (including the initial case that may have initiated herd testing) for the entire

duration of the breakdown incident. In addition, animals in the same group that have been in direct contact (DC) with identified confirmed reactors may also be slaughtered for bTB control (Defra, 2008b), these DCs were not test positive (both under the SICCT and gamma-interferon test) and are purely incidental, therefore are not included in the analysis. Although they may pose certain risk of being infectious to others before being test sensitive.

Introduction source

The following steps describe the process of data filtration combining the Sam's IT system and the CTS database in order to derive the most likely source of disease introduction as defined by effective reactor import.

- Confirmed breakdown herds from Scotland in year 2002 to 2011 which had at least 2 disease cases were selected from Sam's IT system.
- Recurrent breakdowns were removed (i.e. only first breakdown incidence were used, subsequent breakdown episodes were removed to limit the possibilities of residual infection left over from previous incident).
- Subsequent reactor, inconclusive reactor and possible slaughterhouse suspect cases as a result of the breakdown incidence were summarised.
- Their movement records were evaluated by linking corresponding animal passport number in the CTS database.
- Remove homebred (i.e. born on farm) animals that were never moved away from the herd throughout its life history from the dataset. So that only breakdown herds which contains reactors imported (i.e. purchased) from other herds were included. Providing evidence for the likely spread of the disease by cattle-cattle transmission within the herds based on the

assumption that disease introduction were due to the arrival (purchase) of undetected infected animals.

- Identify the time of most recent complete clear whole herd test for each breakdown herds. This was derived from the bTB testing histories in the Sam's IT system (though the majority of the cattle herds in Scotland are regularly tested for bTB, with few exceptions of beef finishing and fattening units that contain no information on previous herd testing due to the quick turnover rate and the fact that no obvious epidemiological risk factors are associated with disease spread. Consequently, these were removed from the analysis).
- Period of at most four years prior to the breakdown date is used as a mandatory clear test date for herds with no previous testing history.
- For breakdown herds where there was an effective risk of movement of reactors since the last clear herd test or in the past four years (whichever is the most recent to the breakdown), the date of disease introduction is defined as the earliest movement date of the reactor with positive *post-mortem* results (i.e. visible lesions or culture positive), or simply the earliest import date of the reactor since the previous clear herd test when all *post-mortem* is negative or unavailable.
- In the case where there are no risky movement of reactors since the previous clear herd test or in the past four years, the date of the previous clear herd test, or four years previously was used as the time of disease introduction.

Population at risk

The mean animal count between period of disease introduction and disease freedom was used as an indication for the number of susceptible animals in each breakdown herd identified under the inclusion criteria. This was obtained from

the “farm population” table in the CTS database which records monthly animal count on each farm holding between January 1999 and March 2013, though only data from 2002 - 2013 were used in this analysis.

Period of disease exposure

The end point of disease exposure is the date when the last reactor was removed from the breakdown herd before movement restriction was lifted following two consecutive clear check tests confirming disease freedom status. This is obtained through linking Sam’s IT system and CTS database using animal ear-tag numbers. Note that the analysis to determine the time of disease freedom is based on off movement dates of all reactors, not just imported reactors as identified in previous exercise in order to infer disease introduction (i.e. disease freedom indicate removal of all infection).

2.2.3 Statistical analysis

An empirical estimate of within-herd incidence rate of bTB for each individual breakdown herd was computed using formula 2.1 after combining all components calculated previously. These estimates (the observed within-herd incidence rate) have different degrees of uncertainty due to variations in the sample sizes (i.e. different herd sizes observed over different length of infection period). The nature of the data means that smaller herds observed over short period of time have greater uncertainty in their transmission estimate compared with larger herds infected over long period of time. If meaningful predictions are to be made from the empirical estimates of within-herd incidence rate, each sample estimate (i.e. estimate from each farm) needs to be weighted towards the mean estimate of the whole population according to the size of observation

(which affects the uncertainty associated with each estimate). The application of generalised linear models provides the opportunity to model this uncertainty accounting for differences as a result of variations in sample size. Therefore fixed effect logistic regression analysis (using “glm” function in the “lme4” package in R) was first carried out to incorporate the different degrees of uncertainty in herd-level incidence rate estimate, assuming binomial distribution of the data, conditional on herd size and duration of infection. The model was fitted to the full dataset comprising reactors, inconclusive reactors and suspect slaughterhouse cases in 110 confirmed breakdown herds over the course of their respective disease episode. The underlying assumption is that there is a true absolute incidence rate across all herds; the different observed incidence rates are consequences of chance or noise due to random error or sampling errors. Moreover the uncertainties in the within-herd incidence rate were assumed to be binomial distributed. The proportion of infection defined as total disease cases in the herd/size of susceptible was used as dependent variable. The independent variables were disease duration, corresponding herd size transformed on the logarithmic scale and farm production type as categorical variable (generalised to five categories: beef, dairy, fattening, suckler and stores).

Despite the inherent uncertainty in each empirical estimate of the within-herd incidence parameter, there may also be natural variations in the rate between different farms (i.e. other than variations caused by noise and sampling error). Therefore, in order to determine whether there are underlying variations in the herd-level incidence rate between individual farm holdings, a random effect logistic regression model was carried out to assess statistical differences in the individual within-herd incidence estimate associated with different farm holdings (using “glmer” function in the “lme4” package in R). Similar to the fixed effect model analysis above, the data were assumed to be binomially distributed with combination of herd sizes and disease duration on log-log and logarithmic

scale respectively, along with production type (categorical) as fixed effect model parameters. Fitted random effects were “farm id”.

A total of 5 logistic regression models were fitted to investigate the potential variation on the within-herd incidence rate between different farm holdings. They are summarised in Table 2.1.

Table 2.1: 1 fixed effect and 4 random effects logistic regression models with different set of model parameters and their respective AIC fit statistics

Mixed models	Model Parameters
Fixed effect logistic	$\log(days)$, $\log(size)$, production type
Random effect logistic (1)	$\log(days)$
Random effect logistic (2)	$\log(days)$, $\log(size)$, production type
Random effect logistic (3)	$\log(days)$, $\log(\log(size))$, production type
Random effect logistic (4)	$\log(\log(days))$, $\log(size)$, production type

These competing models were summarised and the best model is chosen based on the fit statistics: Akaike Information Criterion (AIC). The model with the lowest AIC is chosen as the best model fit and the model outcome with parameter estimate for the chosen model is reported. Associations with a p-value < 0.05 were considered statistically significant. All statistical analyses were performed in SAS software.

2.3 Results

There was a total of 52,051 complete herd tests conducted in Scotland between 2002 and 2011 resulting in 564 unique breakdown incidents from 15,290 herds. Of which 172 were confirmed by the presence of at least one animal (from a group of reactors or slaughterhouse cases under the same breakdown incident) identified with visible lesions at *post-mortem* examination or has obtained a positive culture result from isolating mycobacteria in tissue samples (necessary regulatory requirement to confirm breakdown herds). These 172 incidents included 12 recurrent breakdowns which were subsequently removed (initial breakdown incidents were retained). This gives 160 new breakdown incidences where there has not been a previous disease history in the herd.

In addition, 39 confirmed breakdowns were removed from the resulting dataset that consisted of only 1 identified reactor (i.e. indicating no within-herd spread). There were also 4 herds that were unable to be matched between Sam's IT system and CTS database due to inconsistent location ids. Therefore, the final dataset composed of 117 confirmed breakdown herds with a total of 1,661 slaughtered animals including 1,528 reactors, 90 inconclusives that were slaughtered following the initial and subsequent follow up tests and 43 slaughterhouse suspect cases identified through routine slaughter.

Despite the regulation requirement for 60 days interval testing, the shortest breakdown was resolved in only 43 days (herd depopulated), while the longest lasted almost 3 years (Figure 2.2). However, the majority (75%) of confirmed breakdown incidences ranged from 100 and 300 days. This is due to the current regulation that any confirmed breakdown herds must clear two consecutive whole herd test at least 60 days apart before movement restriction can be removed.

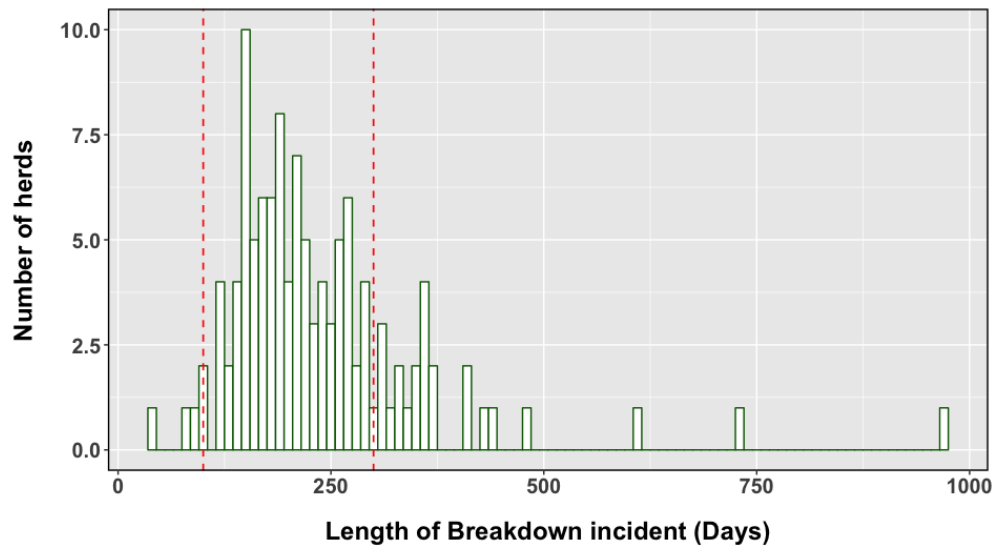


Figure 2.2: Bar chart showing the distribution of length of breakdowns in confirmed bTB farms in Scotland between 2002 and 2011. The red dashed line indicate the 12.5 and 87.5th percentile of the entire data range.

Infected cases

34 breakdowns identified in the analysis (which represents 30% of total breakdown incidences in Scotland) were disclosed as a result of positive regular slaughterhouse surveillance, where animal carcasses have been examined for visible lesion. A total of 90 inconclusive reactor and 1,492 positive reactors were slaughtered for bTB control between year 2002 and 2011.

Plot for the total number of days at risk vs herd size in Figure 2.3 indicates that for short disease exposure time, apart from some larger herds (e.g. more than 500 animals), almost all farms have relatively few infected cases. While some herds under long period of exposure from infection tends to have more disease cases during the breakdown, though this increase is not linear (herds with exposure between 900 - 1300 animal days exhibits the most number of cases Figure 2.3). Generally speaking, there is no resounding pattern between herd sizes and days at risk (e.g. there are also some larger herds with long

duration of infection that has small number of infected animals). However, from epidemiological point of view, larger herds naturally contains more susceptible animals and given an infectious source (i.e. infected and undetected cattle), can potentially contributes to large breakdowns. It is also clear from Figure 2.3 that there are two potential outliers in the study sample. These herds have abnormal number of animals (exceeding 5000), and is likely to operate with separate land parcels in reality but all registered under one main holding in the CTS database. Hence the final analysis of the within-herd incidence rate is carried out with these outliers removed.

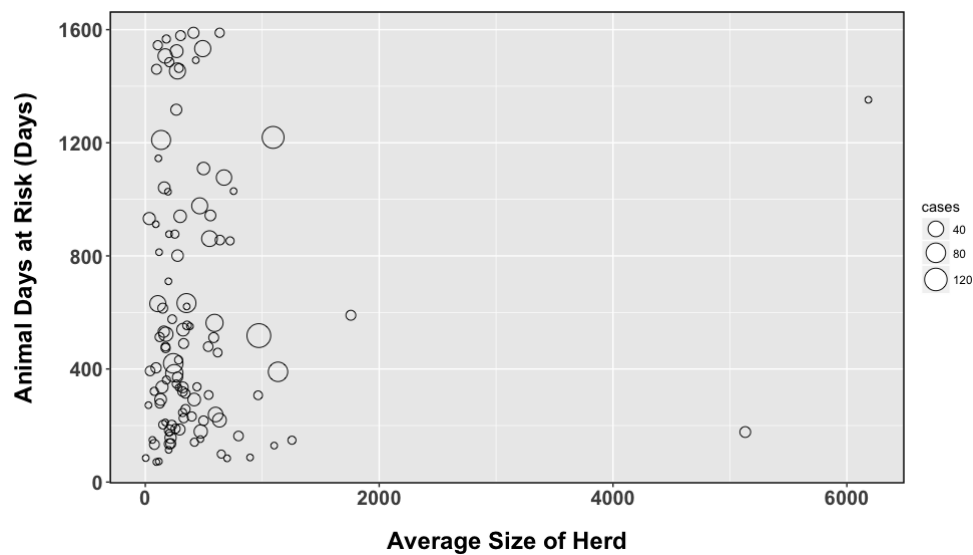


Figure 2.3: Average herd population in relation to the length of infection, where the size of circle represent the size of disease cases during the infection period

Introduction source

654 homebred animals with no previous movement histories were removed from 64 breakdown herds (Figure 2.4). As a result of data filtration, 6 breakdown herds were dropped from the analysis (reactor animals were all home-bred). Disease introduction source in these herds (i.e. non home-bred reactors) may have being transferred onto other herds before the disclosure of breakdown occurred, and therefore were unable to be traced directly from the list of disclosed reactors; or there are exposures to environmental *M. bovis* which was not considered under the assumptions for this study. As a result, this further reduced the dataset to 111 confirmed breakdown herds (as shown in the outmost circle in Figure 2.4). From those, 70 had a complete previous clear whole herd test prior to the breakdown, others were assumed to have been tested at most 4 years before the breakdown date. 22 of the remaining herds had reactors imported after the previous clear herd test but was not identified with visible lesions or positive culture (middle circle in Figure 2.4). If this is the case, the earliest movement date of reactor immediately after the clear herd test was used as point of disease introduction. The other 68 herds had imported reactors which had obtained a positive outcome in the *post-mortem* test, as before, the earliest movement date of the reactor is defined as the source of disease introduction (inner circle in Figure 2.4).

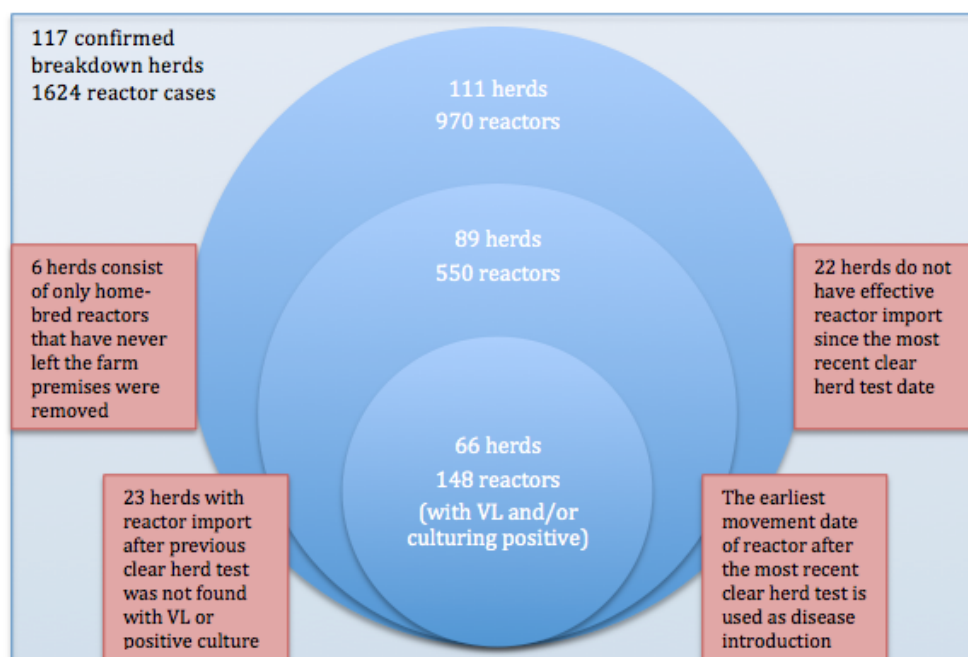


Figure 2.4: Schematic representation showing each step of the data filtration used to identify the point of disease introduction. The outer rectangle indicates the total study sample with each circular region representing the results following the process of data filtration.

Approximately 23% of the farms were clustered towards the lower end of the scale where the within-herd spread was considered to occur 50 to 80 days before being detected by the routine surveillance activity (Figure 2.5). However, in general, more than 50% of herds were detected within a year from the initial proposed infection time. Occasional long-lasting spread beyond 400 days is indicated by a small but noticeable cluster of farms towards the high end of the scale that have an extremely long period of hidden infection.

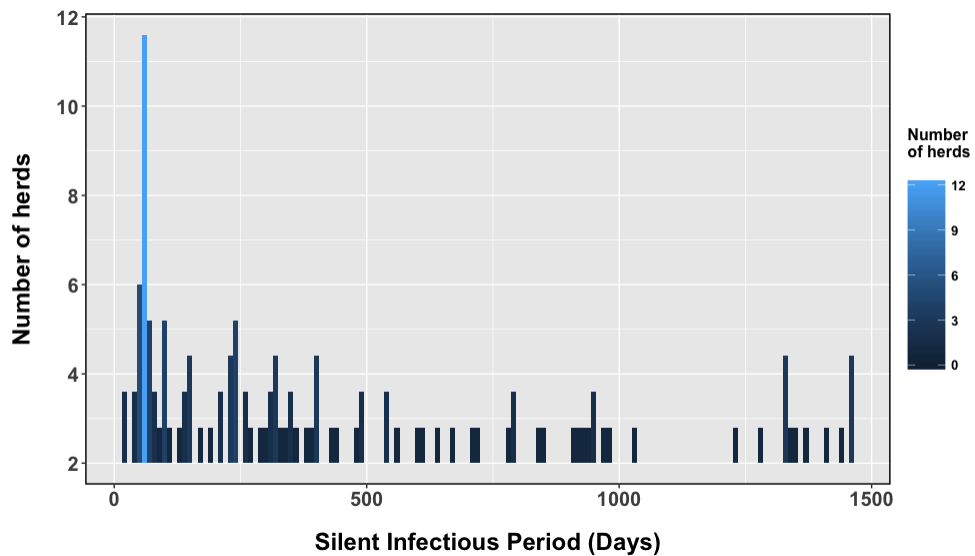


Figure 2.5: Distribution of the length of silent spread (undetected infection) from the (inferred) disease introduction to the initial disease detection

Disease introduction was assumed to be possible after the most recent clear whole herd test. The period during which disease was introduced after the initial clear herd test is presented in an ordered bar chart in Figure 2.6. The disease introduction in breakdown herds without movements from test positive animals were re-adjusted to the point of previous clear herd test. The potential disease introduction in these herds may have been due to animal movements after the clear herd test date but was perhaps removed before the breakdown was detected.

Moreover, Figure 2.6 indicates that the majority of late introductions were from herds with inferred (unidentified) clear test dates.

In summary, out of the 111 herds with inferred disease introduction date, 40% of confirmed breakdowns were caused by movements of cattle located exclusively on Scottish cattle farms prior to detection date. Another 40% of breakdown farms were caused by animal import from high-risk areas of Ireland, Northern Ireland, England and Wales. For the remaining 20%, there was no association with the actual animal movements; they may be the result of residual infections from a previous breakdown incident or that the infection source was removed from the herd before detection was made.

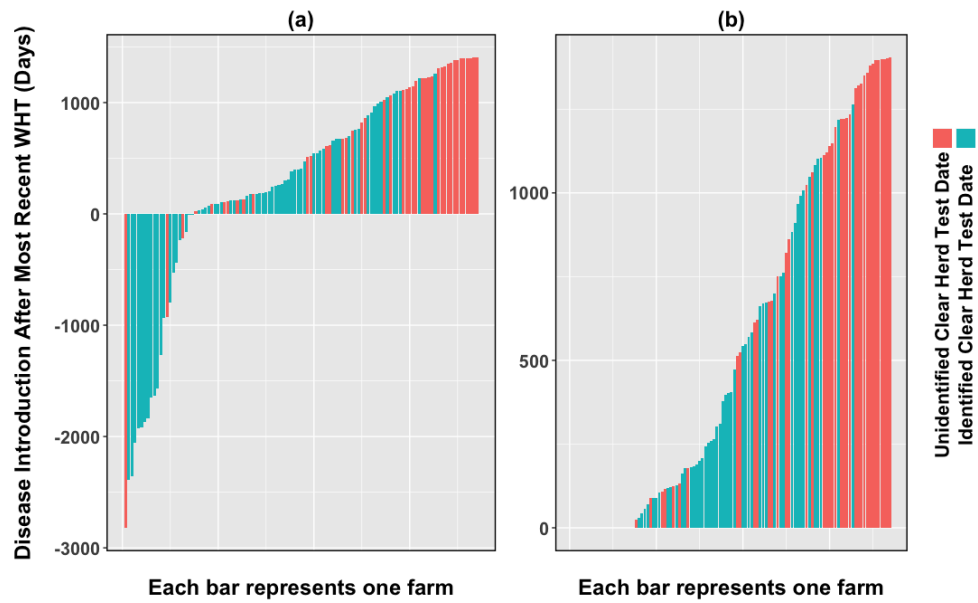


Figure 2.6: The point of disease introduction in relation to recent clear whole herd test a) for herds with reactor imports prior to the clear test date, negative values indicate movements prior to the date of clear herd test. b) after adjusting for prior movements before clear herd test by implying that the date of disease introduction is at least the point of clear herd test date (assuming clear herd test is an indication of disease freedom). The green bars represent herds with identified previous clear herd test prior to the current breakdown, whereas red bar represents artificial mandatory clear herd test date that is four years before the breakdown date

Population at risk

More than half (58%) of the cattle herds are breeding herds (i.e. suckler or producer herds), with the rest composed of a combination of beef-rearer and finishing units (using SAM's IT system classification). The mean herd population size during the disease period ranges from 4 to 6,184 animals, with vast majority of herds (80%) having fewer than 500 animals on average across the disease episode. While small herd sizes are perfectly reasonable, herds with large number of cattle are of great concern and may become inefficient to manage. There are two outlier herds with an average size greater than 5,000 according to records in CTS database, however, detailed investigation revealed that these two herds have a long list of additional land and shared facilities between the infection period, which perhaps suggest that the animals were in fact distributed over a selection of premises rather than under one farm holding. The plot of average herd size against length of OTF recovery indicates that larger herds (a combination of suckler and finishing herds) tend to have slightly longer breakdown periods from the initial point of disease detection (Figure 2.7), though this trend is positive but weak.

Period of disease exposure

The disease exposure period (i.e. from disease introduction to point of disease freedom) ranges from 71 days to 1589 days, and this is compared to the speed that a confirmed breakdown can be resolved (Figure 2.8). The time it takes to recover OTF status in confirmed breakdown herds increases steadily with longer disease exposure period. However, at the individual herd-level, there is no clear indication that longer infection time leads to delayed clearance for movement restriction. Apart from a few large outbreaks in what can be labelled as unusual events due to external factors, majority of farms managed to regain its OTF status in less than a year since the initial breakdown.

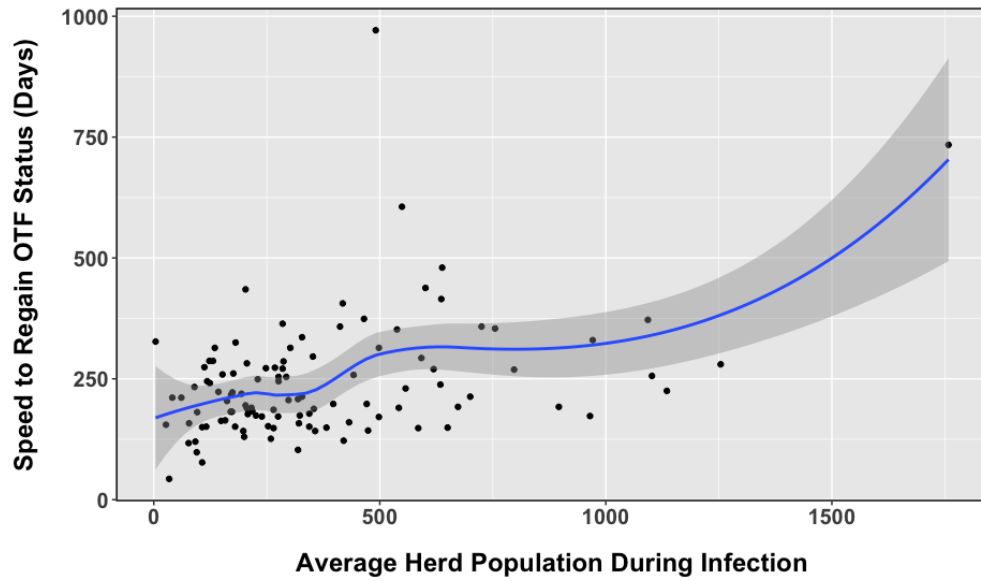


Figure 2.7: Average herd population against the time to regain OTF status and confirmation of clearance from infection from the point of breakdown declaration. Local smoothing is used to map on the data points with standard error represented by the shaded region.

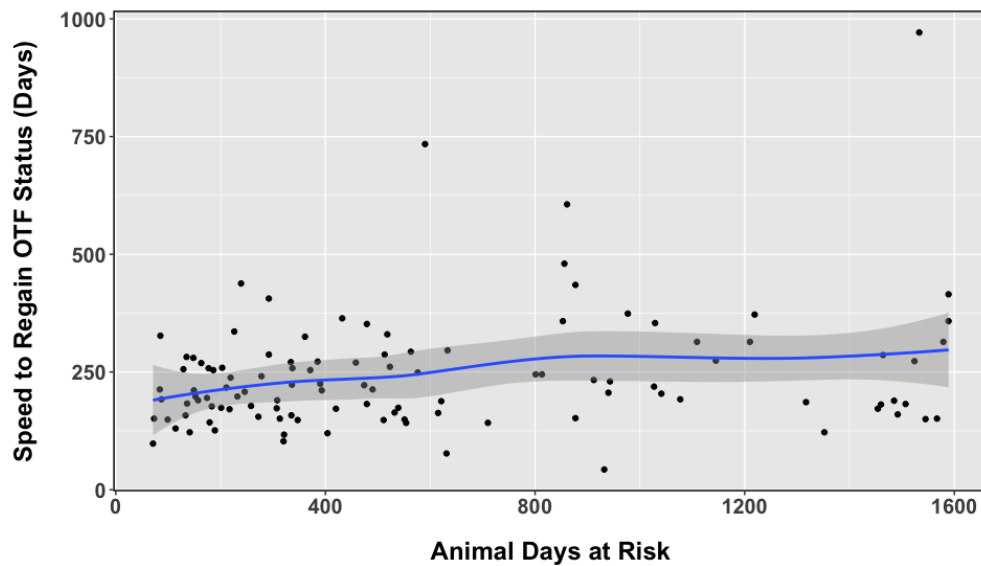


Figure 2.8: Speed of OTF recovery against length of disease exposure in 111 confirmed breakdown farms in Scotland between 2002 to 2012. The regression line is fitted using local smoothing with standard error represented by the shaded region

2.3.1 Within-herd incidence rate

The global mean within-herd incidence rate across the whole of Scotland is 4.51×10^{-7} per cow per month, but the mean rate amongst each individual breakdown herd is 0.0032 per cow per month (as indicated in Figure 2.9 by the dashed line). This means that on average, 3 cows are expected to be infected per month in a herd with 1000 animals after arrival of an initial single case disease introduction in the form of effective high-risk animal movements (Figure 2.9).

A herd with the highest within-herd incidence rate (0.1 per cow per month) had only 4 susceptible animals with 2 identified positive reactors; this extremely small sample size is reflected by the wide confidence intervals associated with large uncertainty in this estimate. Moreover, this herd is a finishing unit that regularly import animals from high-risk areas of Northern Ireland ready for slaughter, it therefore has extremely high rate of turnover, hence identified reactors on this herds are the results of movement only (i.e. not disease transmission within-herd). The analysis on animal movement history confirms this, where the initial reactor was slaughtered before the introduction of the next positive animal, hence there was no overlap between the two cases and therefore no onward transmission. As a result, this herd was removed from the analysis.

It can also be seen from the results in Figure 2.9 that higher within-herd incidence rates (0.01 infected cows per month) were observed in certain herds (mostly suckler herds with two finishing units) in comparison with the others, which suggest that some herds may contain individuals who are potential super spreaders and may play a key role in local transmission of the disease.

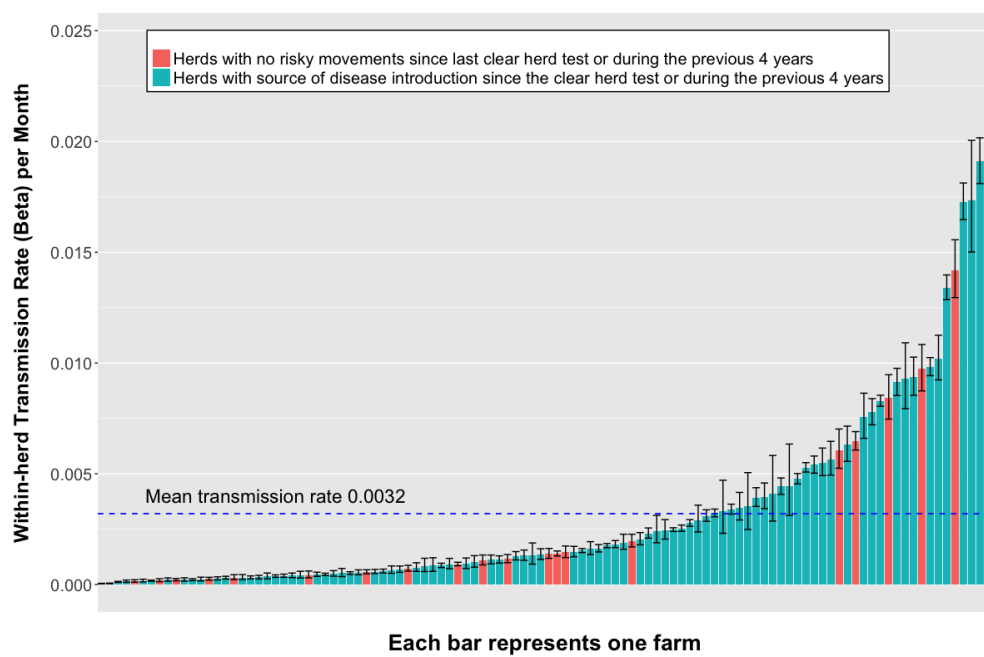


Figure 2.9: Monthly within-herd incidence rate based on inferred disease introduction of bTB in 111 confirmed breakdown herds in Scotland between year 2002 and 2011. The 95% binomial confidence interval is plotted on top of each bar

2.3.2 Statistical analysis

From Table 2.2, the linear mixed model incorporating $\log(\log(days))$ and $\log(size)$ as fixed effect with farm as random effect provides the best model fit to the resulting empirical estimate of within-herd incidence rate, despite the penalising effect of requiring additional random effect parameters. In addition, it is clear that binomial uncertainty alone was not sufficient to explain the underlying variations in the estimate (as indicated by the fixed effect logistic model statistics). And the between farm variation effect is confirmed by the covariance parameter estimate (1.58), which signifies a significant variation in the transmission rate amongst different farm holdings. The production type covariable as a fixed effect was not statistically significant across all the categories (p-value > 0.2) in the logistic regression analyses, and is consequently dropped from the model output.

Table 2.2: Logistic regression models with different set of model parameters and AIC fit statistics

Mixed models	Model Parameters	AIC
Random effect logistic (1)	$\log(days)$	760.5
Random effect logistic (2)	$\log(days), \log(size)$	746.2
Random effect logistic (3)	$\log(days), \log(\log(size))$	746.1
Random effect logistic (4)	$\log(\log(days)), \log(size)$	744.3
Fixed effect logistic	$\log(\log(days)), \log(size)$	2592.1

The statistically significant model parameters were in line with conclusions reached from the empirical analysis. The estimates from the model parameter indicate that larger herds (i.e. $\log(size)$) were associated with lower within-herd

incidence rate, while longer disease exposure period (i.e. $\log(\log(\text{days}))$) tends to increase the overall rate of within-herd incidence (Table 2.3).

Table 2.3: Random effect logistic regression model parameter estimates for fixed effect terms

Effect	Estimate	Standard Error (SE)	p-value
Intercept	-5.9633	2.0092	0.0029
$\log(\log(\text{days}))$	3.4393	0.9618	0.0003
$\log(\text{size})$	-0.7187	0.1702	< 0.0001

The results can be used to estimate the proportion of infected (p) at each farm :

$$\ln\left(\frac{\rho}{1-\rho}\right) = -5.96 + 3.44 \times \log(\log(\text{days})) - 0.72 \times \log(\text{size}) + \text{farmRE} \quad (2.2)$$

Where ($\rho = \text{diseasedcases}/\text{totalsusceptible}$), is assumed to have a binomial distribution and the farm random effects follows a normal distribution with zero mean and variance of 1.58 on the logit scale (i.e. $\text{farmRE} \sim N(0, 1.58)$).

In reality, within-herd incidence rate was unlikely to be the same for different durations. In fact the sigmoid shape of the logit function is already quite well matched to relating disease duration and rate of within-herd incidence (i.e. incidence rate does not increase linearly with days). It increases gradually at first, then more quickly before eventually slows down. The model diagnostics did not indicate a cause for concern and residuals appears to be randomly scattered. A mean variance inflation factor (VIF) of 1.78 was calculated amongst variables in

the logistics regression model (as a rule of thumb VIF greater than 10 indicate a problem of collinearity), indicating no issues with variable collinearity.

2.4 Discussions

This chapter presents an empirical analysis that estimates the local rate of incidence of bTB amongst Scottish cattle herds with confirmed infection. The underlying methodology apportions incidence rate evenly across the disease period and therefore represents an overall rate of incidence at individual herd-level. Although this rate does not reflect the true dynamics of within herd transmission through time, the empirical data analysis offers a unique way to obtain insight on the local spread of bTB in areas with low incidence and the results can be used to compare with outcome from conventional models to assess its validity. It may also be used to assist on epidemiological model parameterisation in diseases that are of similar nature.

Scottish cattle herds, with no evidence of an established wildlife reservoir as a transmission source, are used as a basis to estimate within-herd incidence rate in low incidence areas. The comprehensive Sam's IT system and the CTS database comprising bTB testing histories with diagnostic results and detailed cattle movement record provided a unique opportunity to identify the most likely source of infection leading to the quantification of herd-level incidence rate, and offers the opportunity to assess the nature and degree of variation in the within-herd incidence rate between infected cattle herds. The two main objectives of this study were to quantify the within-herd incidence rate of all confirmed breakdown herds in Scotland tested between 2002 and 2011, and construct a realistic empirical sampling distribution of the within-herd incidence rate given

the size of the farm. These findings can also be used to parameterise dynamical models of disease propagation.

Overall, one of the most important findings was that there is an inherent variation in the within-herd incidence rate between different farm holdings. This may be the result of different herd management practices (Goodchild and Clifton-Hadley, 2001; Pfeiffer, 2005), the nature of herd production type / dynamics (Bessell *et al.*, 2012b) or the existence of ‘super-spreading’ animals persistently shedding bacteria allowing efficient transmission to susceptible cattle population (O’Hare *et al.*, 2014). Advanced or generalised disease within the herd would be expected to promote cattle-cattle transmission. It is not known which animals transmit the most, although recent whole-genome sequencing and social-network analyses of bTB infection support the existence of a ‘super-shedder’ animals (Santos *et al.*, 2015; Gardy *et al.*, 2011). There are anecdotal field reports of ‘super-shedder’ cows in the UK cattle herds (Skuce *et al.*, 2011) and some advanced mathematical models fit the observed field data better when a super-shedder is invoked (O’Hare *et al.*, 2014), but there is a lack of direct evidence to support the claim.

Reactors in 58 breakdowns (52%) include homebred and purchased animals in the study sample, while further investigation on the available genotype data from culturing of bacteria revealed that some of the homebred reactors in this study shared the same spoligotype (molecular typing method used to identify bacterial isolate) with the identified disease introduction source, providing further evidence for cattle-cattle transmission within-herd. However, the lack of genotype data on *M. bovis* culture makes it difficult to analyse the situation for every herd. Previous evidence shows that the spoligotype of *M. bovis* isolate tends to be geographically localised (Smith *et al.*, 2006a). Northern Ireland (NI) province-wide animal-level sampling for *M. bovis* genotyping also indicates similar trends

(Skuce *et al.*, 2010). A pivot table of herd by *M. bovis* genotype identified that most of the largest herd breakdowns yielded only one pathogen genotype. This could indicate extensive within-herd cattle-cattle transmission, repeated exposure to point source possibly including contact with infectious wildlife or the existence of super-shedder cows or some combination of these (Skuce *et al.*, 2010). It will be important to investigate, and mitigate where possible, the risk factors associated with such herds and separate those herds where the locally-fixed genotype(s) appear from those which have clearly received genotype(s) via purchase or import, thus providing a unique opportunity to index within-herd spread, should it occur. Other cases of on-farm transmission have been documented in the literature. For instance, an outbreak identified in Netherlands was triggered by the import of a single infected animal which had generated an additional reactor detected 392 days later (Fischer *et al.*, 2005). Also the purchase of a single infected animal resulted in eight further confirmed reactors identified over a 2-year period in a New Zealand dairy herd. These studies have shown that there is clear evidence of onward transmission after disease introduction especially where infections were not detected rapidly (Gopal *et al.*, 2006). With intensive trading between Scottish cattle farms (Volkova *et al.*, 2010) and high frequency of import from high-risk areas (Gates *et al.*, 2013), there is a danger that the infected animals will be sold to other herds leading to secondary bTB outbreaks prior to detection through disease surveillance. Targeting investigation and control at those cattle / herds that are most highly connected in the contact networks should be cost-beneficial.

Length of disease exposure and point of disease introduction

Gilbert *et al.* (2005) reported that movement of cattle from areas where bTB was common to areas where bTB is considered rare was the best predictor for the introduction of bTB into a naive geographical area. The assumption that disease introductions were caused by animal movements may be a reasonable

one, however we recognise that other routes of introductions such as contiguous spread (Wolfe *et al.*, 2010), unidentified wildlife source (Gates *et al.*, 2013) and other external force of infection were also likely (Johnston *et al.*, 2011). Further investigations into these risk factors may shed additional insight on the sporadic outbreaks within Scotland. Also for simplicity, the classification for the point of disease introduction was limited to at most four years prior to the breakdown date. This is because routine surveillance in Scotland prior to 2012 are based on 4-yearly testing, it is unlikely for normal cattle premises be exempt from whole herd testing for more than 4 years.

In addition, when calculating the length of infection, it was impossible to know the exact exposure time for each animal in each herd, due to the fact that little knowledge is known about the exact time when each animal was infected and once they became infected whether or not they are infectious. Therefore, the analysis uses herd-level exposure rate to estimate an overall spread rate of the disease within the herd rather than transmissions at animal level. However, even at herd-level, the disease exposure time was probably over estimated due to the uncertainty in the determination of the time of disease introduction. When several reactor animals were identified as potential introductory source, the earliest movements were used as the date of disease introduction. This is to over compensate so that the herd was said to be free from disease at least up to that point. Moreover, whenever possible, movement dates of reactors with positive *post-mortem* results were selected as effective time of disease introduction in favour of negative or no *post-mortem* diagnostics. This is due to the imperfect test sensitivity, which means that certain percentage of reactors may be false positives, and those with either visible lesion or positive culture are more likely to be true bTB cases compared with reactors with a negative *post-mortem* outcome. On the other hand, it has been suggested that in practice, the culturing effort typically stops once an animal was successfully cultured in a multi-reactor herd,

hence, giving an incomplete set of *post-mortem* diagnostics for the list of identified reactors. Based on this classification criterion, disease introduction may be over-estimated leading to a longer disease exposure period, which results in under-estimation of the within-herd incidence rate.

The results from the multivariable logistic regression model revealed that herd size was negatively associated to the within-herd incidence rate. Herd size as a significant factor relating to herd breakdowns has been found repeatedly in previous research (e.g. Skuce *et al.*, 2012; Bessell *et al.*, 2012b; Olea-Popelka *et al.*, 2004; Byrne *et al.*, 2014). Some suggest that larger herds often have a larger geographic footprint, which may expose them to greater environmental risk factors (e.g. wildlife reservoir) and will also expose them to more neighbours (the risk of contiguous spread), subsequently leading to an increased risk of breakdown (Byrne *et al.*, 2014). However, there was no evidence to suggest that herd size is related to the within-herd spread after initial disease introduction. Due to the way that the within-herd incidence rate was calculated under the current study (i.e. within-herd incidence rate is inversely proportional to the number of susceptible animals), it is not surprising to see the negative effect associated with herd size, because in order to maintain a higher within-herd incidence rate in larger herds, a substantial number of reactors needs to be detected. Given the slow spreading nature of bTB accompanied by intensive surveillance activities, this is rarely the case. Studies have shown that larger herds are more likely to have at least one animal with disease and as herd size increases, the probability of detecting at least one case also increases, herds of different sizes are therefore at different risks (Vial *et al.*, 2011). Though the overall observed size distribution of bTB-affected herds seems to suggest that animals pose identical risks (Bourne, 2007). One suspicion frequently associated with larger herds has been that the number of contacts, and hence the probability of transmission increases, with the number of animals in an epidemiological group (Conlan *et al.*, 2012). Though strictly speaking,

direct contact often depends on the herd stocking density and this is a subtle, but different quantity in relation to herd size. A case-control study conducted by Reilly and Courtenay (2007) demonstrated that higher stocking density was associated with reduced risk of transient and persistent bTB. Perhaps due to small sample sizes, farm production type was not statistically significant in the current study, though their importance in the epidemiology of bTB transmission should not be overlooked. Other studies argued that herd production type and size is often linked to management-related risk factors including trading habits, feeding regime and herd turnover rate, which could all have a strong influence on the within-herd transmission dynamics (Vial *et al.*, 2011; Olea-Popelka *et al.*, 2008; Alvarez *et al.*, 2012; Adkin *et al.*, 2016).

Another important predictor for the within-herd incidence rate is the length of the disease exposure period. It has always been known that bTB incidence increases with age due to the cumulative exposure to either environmental *M. bovis* or previous direct contact with disease dissemination source (Menzies and Neill, 2000). It was demonstrated in a NI study that repeated exposure to point source(s) either through direct contact with super-shedder cow(s) and/or infectious badger(s) could result in large number of reactors within-herd, often with same (or very similar) genotypes (Skuce *et al.*, 2010). Hence it can be argued that in a bTB infected herd, the longer the exposure time to potential infectious source, the higher the probability of within-herd transmission. Although a study of meerkat social group analysis by Drewe (2010) illustrated that exposure time was less important than social interaction in influencing TB risk. The study (using social network analysis and diagnosis of TB) concluded that due to the stable social structure, infection appeared to spread locally within clusters of interacting individuals, while some animal behaviour was more risky than others (Drewe *et al.*, 2011). Another New Zealand study investigating transmission of *M. bovis* between uninfected and infected (wild) feral pigs also made similar

conclusion that within-species transmission was probably insufficient to sustain infection in the wild, and the high prevalence of TB in feral pigs is more likely as a result of transmission from other routes or hosts (Nugent, 2011). However, it is not clear how relevant these studies are to cattle-cattle transmission, since *M. bovis* is not particularly host-adapted to pigs or meerkats and this is known to influence the efficiency of within-species transmission (Smith *et al.*, 2006a). In addition, the strain of *Mycobacterium tuberculosis* identified in meerkats (*M. suricattae*) are different to that of *M. bovis* found in cattle (Parsons *et al.*, 2013).

There are likely to be other factors that can affect the within-herd incidence rate other than herd size and length of exposure, such as herd type, farm management practices, cattle behaviour, weather and climate conditions (Lahuerta-Marin *et al.*, 2015; Reilly and Courtenay, 2007; Skuce *et al.*, 2011; Wint *et al.*, 2002). For instance, the age profile and contact patterns established within beef, dairy and mixed herds are likely to be quite different. Furthermore, there tends to be limited contact between dam and progeny in dairy herds, compared to beef herds where significant amount of contact may occur between adults and calves (Defra, 2000). Defra project SE3003 showed that dairy herds have a higher ‘transmission coefficient’ than beef herds due to their longevity and more intensive management system, which often results in closer confinement (Defra, 2000). Other management-related risk factors includes feeding regime, herd turnover rate and production stress (Vial *et al.*, 2011). Weather and climate has been linked to geographical and temporal variation in bTB incidence (Wint *et al.*, 2002). A study has showed that climate may contribute to the geographical localisation of bTB in south-west England and west Wales (King *et al.*, 1999), though direct causation is always difficult to establish in these kind of studies. Data relating to these factors were not available in the CTS database, and could be introduced in the future to enhance disease management and control strategies.

Study limitations

Throughout the analyses, there were many challenges when using the CTS database and the Sam's IT system. First, surveillance results from outbreaks in the Sam's IT system are stored under the main farm CPH number regardless of whether cattle are housed on that location or on other uniquely identified land parcels operated by the same cattle business (Gates *et al.*, 2013). Furthermore, farmers that have registered for "linked holding" status are not required to report the movements of cattle between land parcels under the same occupancy holding (Orton *et al.*, 2012). This leads to many animals been excluded from the analysis because of inconsistency in their present location. In addition, there were several difficulties encountered when matching between Sam's IT system and CTS databases. These problem were often due to non-matching record of unique location identification number of the farm, since Sam's IT system uses different standard to record locations in comparison with CTS. Also animal passport number are different across the two databases, this may cause farms or animals been unidentifiable. During the analyses, we have encountered approximately 5% of herds with non-matching location number, these were investigated and corrected before been included into the analysis.

In addition, under our classification on the disease introduction, 24 confirmed breakdown herds had inferred introductions due to "placement" moves prior to 2002. These were administrative entries when CTS database was first updated in year 2000 to support disease control activities. As a result, a series of mandatory movement dates were used by administration to account for animals that already existed on a farm previously. Also as with any data source, CTS are subject to errors and omissions. Where there are missing information, animal movements are automatically inferred based on information available, this

could cause a problem if large proportion of records are estimates rather than actual. Therefore, we cannot reasonably rely on movement entries prior to 2002, as there are substantial arbitrary entries to the database that does not correspond to real movement dates. Disease introductions for the majority of these herds were defined to be 4 years prior to the breakdown dates rather than due to animal movements.

Another potential limitation of this analysis was that the length of the infectious period for a premises was ambiguous and difficult to determine. Therefore, it is possible that the infectious period may be longer or shorter, rendering a smaller or larger (in the latter case) within-herd incidence rate. Given the slow spreading nature (i.e. low within-herd incidence rate) of bTB (Bourne, 2007; OIE, 2009), and the large sample size in the study, this was considered to have only a minor consequences on the resulting distribution of the incidence rate.

A more comprehensive investigation of transmission chains in cattle contact networks may be conducted should more genetic and epidemiological data be made available. The analysis of cattle movement and bTB test history data alone may not completely capture transmission dynamics and may lead to an underestimation of the potential for disease spread, especially the extent of local spread via cattle. Investigating the spatial and temporal pattern of disease clusters (including *M. bovis* genotype clusters) may help to identify those local risk factors which contribute most to the ongoing transmission producing the cluster and to identify the sources of infection.

2.5 Conclusion

Conventional bTB transmission models rely on complex model structure and difficult fitting procedures to estimate the within-herd dynamics of disease spread. The empirical analysis based entirely on previous animal movement and surveillance data provides additional insight in the local transmission of bTB at the individual herd-level. Based on the study findings, within-herd incidence rate is higher when duration of infection is long and lower in herds with large size; it also has inherent variability between different farms. The distribution of within-herd incidence rate is highly skewed and tuberculin reactors tend to cluster within herds. This suggests that a relatively small number of herds contribute disproportionately to the overall number of reactors. It implies that there are likely to be converging risk factors for those herds, which support cattle-cattle transmission and which may also include susceptibility risks. The concept of ‘super-shedder animals’ are well documented, but evidence of significant herd-level characteristics (e.g. management practices and farmer trading behaviour) contributing to bTB spread may lend support to the suggestion of ‘super-spreading’ herds. Thus, when it comes to implementing disease control policies, targeting of appropriate control measures to relatively few herds (‘super-spreaders’) could lead to a disproportional and cost-efficient benefit.

Although the within-herd transmission rate of bTB is always difficult to estimate due to the lack of empirical data on the within-herd structure and transmission dynamics, current analysis represent a direct (data-driven) approach to the estimation procedure. By calculating the average number of expected infections on farms with any given size and period, it is possible to provide better guidance to farmers, veterinarians, and policy makers on the optimal strategies for disease control and prevention, thus allocating resources more effectively to

areas associated with high potential of infection. In addition, this study can also be linked with traditional simulation models to parameterise the within-herd bTB transmission process and evaluating the potential impact of the national disease surveillance programmes.

Chapter 3

Stochastic simulation modelling of bTB interventions

3.1 Introduction

Simulation models have been extensively used in veterinary science to increase understanding of disease epidemics and to investigate control strategies, such as for bovine spongiform encephalopathy (BSE; e.g. Anderson *et al.*, 1996), foot and mouth disease (FMD; e.g. Keeling *et al.*, 2001; Morris *et al.*, 2001), avian influenza (e.g. Sharkey *et al.*, 2008) and classical swine fever (e.g. Boklund *et al.*, 2009). These models are often used to support the decision-making process for future planning of disease prevention and eradication programmes by simulating plausible real-world scenarios (e.g. the presence and spread of an infection in an animal population) (Merl *et al.*, 2009; Szmaraagd *et al.*, 2010; Brooks-Pollock *et al.*, 2014).

Bovine tuberculosis (bTB), owing to the long timescales associated with

the disease (Brooks-Pollock *et al.*, 2014), the latent nature of infection (Conlan *et al.*, 2012), the ambiguity in the transmission pathways (Fischer *et al.*, 2005), the potential contribution from wildlife reservoir (Donnelly *et al.*, 2006) and the effect of complex and changing control policies (Defra, 2010), poses difficult challenges in simulating the disease epidemics. However, the availability of data from the Cattle Tracing System (CTS) and historic surveillance testing results (Sam’s IT system) has enabled detailed investigation of bTB infections that are spread by the movement of cattle and interactions with infected wildlife reservoir (Green *et al.*, 2008; Woolhouse *et al.*, 2005). These datasets offer an opportunity to explore the impact of spatial and individual heterogeneities on the course of an bTB epidemic and the importance of these variables for the design of appropriate disease control programmes.

It is generally accepted that there are two main components of the transmission of bTB: within-farm and between-farm transmission (Carrique-Mas *et al.*, 2008). Given the chronic nature of bTB and the limitation of current diagnostic tests, the S-E-T-I (Susceptible, Exposed (or Latent), Test sensitive, infectious) state transition model has been suggested as the most relevant model formulation to capture the infection dynamics within each farm (O’Hare *et al.*, 2014; Fischer *et al.*, 2005; Barlow *et al.*, 1997; Conlan *et al.*, 2012). While between-farm transmission is dependent on the connectivity and association-frequency of the underlying contact structure (Woolhouse *et al.*, 2005; Nickbakhsh *et al.*, 2011), both type of transmission can involve direct cattle-to-cattle transmission as well as indirect transmission from the farm environment, which may include the effect of contaminated pasture and infected wildlife (Brooks-Pollock *et al.*, 2014).

When simulating disease epidemics such as bTB, as well as modelling disease transmission dynamics, it is also informative to incorporate disease intervention strategies. These are often based on current control programmes. In developed

countries such as UK, the majority of infected cattle show no clinical signs, current surveillance are generally based on routine *ante-mortem* testing of individual cattle using the single intradermal comparative tuberculin tests (SICCT) at intervals determined by the herd-level risk and *post-mortem* examination of all bovine carcasses at abattoirs for lesion consistent with bTB (Pavlik, 2006; Radunz, 2006). Both surveillance methods are considered good herd-level screening tools in regions where the prevalence of infected cattle is generally high (de la Rua-Domenech *et al.*, 2006). However, limitations on individual animal sensitivity have been highlighted as the main barrier to eradicating the disease, especially in low incidence regions (Reviriego Gordejo and Vermeersch, 2006). Currently, the estimated relative sensitivity of the SICCT used in routine herd surveillance in the UK ranges from 51% - 80% (specificity around 99%), while from a meta-analysis of published literature the slaughterhouse meat inspection (SMI) has sensitivity and specificity of approximately 69% and 100% (Downs *et al.*, 2011; O'Hare *et al.*, 2014), though in reality under field conditions, the sensitivities are likely to be lower (Broughan *et al.*, 2014).

Studies have been conducted to evaluate the efficacy of different routine surveillance strategy of bTB in combination to the regular slaughterhouse inspection (Fischer *et al.*, 2005; Van Asseldonk *et al.*, 2005; Bessell *et al.*, 2012a), though these models purely assume a constant background risk of disease introduction and does not incorporate a dynamic system of transmission via animal movements nor does it have the flexibility to adjust the timings of each herd tests. Previous simulation models of bTB have been developed that combine disease transmission dynamics and herd-level testing strategies with a view to informing government policy (Barlow *et al.*, 1997; Conlan *et al.*, 2012; Brooks-Pollock *et al.*, 2014). However, extant models either focus on model parameterisation or fails to explore the effect of different routine herd surveillance scenarios on the long-term trends of the disease epidemic. In this chapter, I present a dynamic

stochastic model to simulate bTB infection within theoretical farm contact networks using pre-existing disease parameters estimated from previous studies. The model mimics the disease situation after the introduction of one infected animal into a herd. At the farm scale the model incorporates stochastic transmission of infection within-farm and between-farms, maintenance of infection in the environment (through established wildlife reservoir and external disease introductions), regular slaughterhouse surveillance activity and different routine herd testing protocols to practice and simulate control programmes in order to determine the long term consequences of alternative routine surveillance strategies. Specifically, the analyses aims to address the following issues:

- **Random vs scale-free networks.** Using theoretical contact network structures to model bTB spread rather than purely through background risk of disease introduction. Evaluate the impact on disease spread and detection between random and scale-free network structure on the course of the epidemic.
- **Is timing and intensity of the routine herd test important in disease detection?** Adjust timings of routine herd tests to evaluate the potential benefit in disease detection by testing herds within short period of time.
- **Will increasing routine herd test frequency result in rapid disease detection?** Evaluate the impact on the overall epidemic (in terms of size of infection and time of initial detection) while using different routine test intervals and determine whether slaughterhouse surveillance alone is sufficient to maintain low levels of infection.
- **Consider what would happen should the epidemiological situation change and bTB becomes more transmissible.** This involves varying

the within-herd transmission parameter and different levels of background risks of disease introduction (including from wildlife sources).

- **Consider the scenario where a better diagnostic test is available to detect bTB.** Evaluate alternative diagnostic tests with different levels of test sensitivity (on individual animal level).

3.2 Materials and methods

3.2.1 Model formulation

The model was designed to simulate the change in disease state of individual cattle in a population of herds on a daily basis and to evaluate and identify likely consequences due to changes in routine surveillance strategies in the face of ongoing disease threat. To account for both within-herd and between-herd transmission of bTB, a herd-level (premises based) stochastic meta population model was used. Each outbreak was simulated by introduction of one infectious animal in only one herd, which in turn can lead to a chain of infected animals that are spread between herds by movement of cattle. The within-herd transmission process was modelled using a state transition model with disease classes representing susceptible (state S), incubating (exposed, state E and test sensitive, state T) and infectious (state I) animals. The spread of infection between herds was considered to be due to movement of infected animals to uninfected herds (by means of trading). Selling and buying animals in the model was based on randomly generated contact networks with pre-determined network properties.

Following infection, detection of the disease was through regular monthly slaughter where infected animals can potentially be slaughtered and identified at

the *post-mortem* meat inspection and the addition of a whole herd bTB-test on a routine basis at pre-determined cyclical time points. At the end of each time-step, the disease status of each animal was aggregated at herd-level, and detected premises were noted along with the time of detection. Therefore, the output of the model for each of the evaluated surveillance strategies was a time series of the total number of infected animals in each disease class and the number of infected and detected farms for each time step. With this information, quantities such as time until initial detection, prevalence in detected herd and the number of infected herds at the moment of detection could be calculated.

Although there were no distinct profiles that distinguish individual animals in a herd, in the herd-level (premises) based model, each individual premises was treated as a separate item with a number of herd profile variables (i.e. farm characteristics). These individual premises were subject to processes according to the value of their profile variables and the interaction with other premises in the network. A similar structure was also used in a simulation study by Kooijman (1994). The dynamics of the premises in the model were described by herd size (i.e. number of susceptible and infected animals), monthly slaughter rate, monthly movement rate, routine herd test dates and potential contacts with other premises. The model was stochastic to be able to evaluate the variation in time until disease detection and the outbreak size, which is included in the measure of risk. The choice of an herd-level based approach was driven by the complex and slow infection dynamics of bTB interacting with the dynamics of the host (cattle) population (Fischer *et al.*, 2005).

The model was programmed in R (R, 2012) and consisted of three components, which are described in more detail in the following sections: (1) within-herd transmission, (2) between-herd transmission and (3) disease detection through regular slaughter and routine herd tests.

3.2.1.1 Within-herd transmission process

As herd size and density are known to be correlated to persistent infection within farms (Brooks-Pollock and Keeling, 2009), the spread of the disease between individuals was assumed to be density dependent and is proportional to the number of infectious (I) and susceptible (S) cattle in the herd at any given time, with the susceptible (S) cattle becoming exposed (E) through infectious contact within the herd at rate of β (transmission coefficient). External factors can also contribute to new infection that may include for example, high risk cattle movements, contiguous spread from neighbouring herds or the presence of a wildlife reservoir. Similar to other bTB models (Conlan *et al.*, 2012; O'Hare *et al.*, 2014), these external factors were incorporated into the model via a single external force of infection, α . Therefore, the number of new infections per unit time is given by the density-dependent transmission with infection occurring at base rate βSI , and an additional external infection at rate αS .

Following infection, the disease develops through a number of stages represented by exposed (E), where an animal is infected but neither tests positive for the disease nor infects other cattle, test sensitive (T), where the animal can test positive for bTB but is not yet infectious; and infectious (I), where the animal is both test sensitive and infectious. Once an animal becomes infectious, it remains so until it is detected, at which point all infected animals in the herd would be removed and replaced by susceptible animals at the next time step. The possibility that cattle are immediately infectious were not considered (Conlan *et al.*, 2012), as evidence for this is largely experimental (Kao *et al.*, 2007). The model explicitly implies that exposed cattle become test-sensitive at a rate σ and then infectious at a rate γ .

The SETI model structure for each time step is described by the following

system of ordinary differential equations (ODE):

$$\frac{dS_i}{dt} = -\beta S_i I_i - \alpha S_i \quad (3.1)$$

$$\frac{dE_i}{dt} = \beta S_i I_i + \alpha S_i - \sigma E_i \quad (3.2)$$

$$\frac{dT_i}{dt} = \sigma E_i - \gamma T_i \quad (3.3)$$

$$\frac{dI_i}{dt} = \gamma T_i \quad (3.4)$$

where subscripts denote the different premises in the model. Transition between states were modelled by transition probabilities (described in later sections), which were fixed based on previous estimates of the average duration of each state.

The SETI model described above was solved by using a stochastic process with a fixed number of time steps.

Current literature of bTB has suggested that infectious individuals may be infectious only intermittently (Barlow *et al.*, 1997), and that the latency period of the disease is largely uncertain (Kao *et al.*, 2007). In order to account for any periodic loss of infectiousness and the uncertainties in transitions between model states, the discrete-time stochastic process was used to simulate the disease transmission dynamics within-herd on a day-to-day basis. The method incorporates uncertainties in the transmission by sampling from a binomial distribution with specific epidemiological parameters to determine a change in disease status of an individual animal.

In the individual (premises) based model, the disease dynamic variables for each premise at time t are the number of susceptible, $S(t)$; the number of exposed, $E(t)$; the number of test sensitive, $T(t)$ and the number of infectious individuals, $I(t)$. The model assumes constant herd size across all premises, where animals

removed (i.e. through culling or outward movement) were immediately replaced by susceptible animals, so that $S(t) + E(t) + T(t) + I(t) = N$, where N is the constant herd size.

To characterise the disease transmission, a mass-action term βSI for the transmission function was adapted (McCallum *et al.*, 2001) that can be interpreted as a compound stochastic process in which the infected and susceptible hosts mix completely with each other and infectious contact occurs randomly (i.e. according to a Poisson process) with a constant encounter rate within the herd (Merl *et al.*, 2009). The process also assumes homogenous susceptibility for each individual.

Hence, the SETI model formulation leads to a natural discrete time approximation for the numbers of exposed (\tilde{E}), test sensitive (\tilde{T}), and infectious (\tilde{I}) arising in the unit time interval from t to $t + 1$. Holding the total number of infected individuals, I , constant and integrating both sides of Eq. 3.1 over a unit time interval between t and $t + 1$ gives

$$\begin{aligned} \int_t^{t+1} \frac{1}{S} dS &= \int_t^{t+1} (-\beta I - \alpha) dt \\ \left[\ln(S) \right]_t^{t+1} &= \left[(\beta I - \alpha) t \right]_t^{t+1} \\ \ln \left[\frac{S(t+1)}{S(t)} \right] &= (-\beta I - \alpha) \\ S(t+1) &= S(t) \left[e^{(-\beta I - \alpha)} \right] \end{aligned} \tag{3.5}$$

so that the fraction of susceptible individuals surviving throughout the unit time interval is $[e^{(-\beta I - \alpha)}]$. When viewed as a discrete time stochastic process, where the mean number of remaining susceptible individuals is given by Eq. 3.5, the mean number of newly exposed (infected) occurring between time t and $t + 1$ is

therefore given by

$$S(t) \left(1 - \left[e^{(-\beta I - \alpha)} \right] \right).$$

This immediately implies that the new exposed individuals (\tilde{E}) at time $t+1$ can sensibly be given as

$$\tilde{E} \mid S(t), I(t) \sim \text{Bin}(S(t), p_{\text{expo}}(I(t), \beta, \alpha)), \text{ where } p_{\text{expo}}(I(t), \beta, \alpha) = 1 - [e^{(-\beta I(t) - \alpha)}]$$

and $\text{Bin}(n, \pi)$ is the standard binomial distribution with number of trials n and success probability π . Similarly, by integrating Eq. 3.3, the numbers of new test sensitive and infectious individuals occurring between time t and $t+1$ can be described by

$$\tilde{T} \mid E(t) \sim \text{Bin}(E(t), p_{\text{test}}) \quad (3.6)$$

$$\tilde{I} \mid T(t) \sim \text{Bin}(T(t), p_{\text{inf}}) \quad (3.7)$$

where $p_{\text{test}} = 1 - e^{-\sigma}$ and $p_{\text{inf}} = 1 - e^{-\gamma}$. The forward dynamics for the total numbers of exposed, test sensitive and infectious individuals are therefore

$$S(t+1) = S(t) - \tilde{E} \mid S(t), I(t)$$

$$E(t+1) = E(t) + \tilde{E} \mid S(t), I(t) - \tilde{T} \mid E(t)$$

$$T(t+1) = T(t) + \tilde{T} \mid E(t) - \tilde{I} \mid T(t)$$

$$I(t+1) = I(t) + \tilde{I} \mid T(t)$$

This discrete time approximation assumes a particular ordering of events, namely that animal becomes exposed (infected) first, followed by test sensitive, to infectious and then finally to potential new infection. Simulation studies indicated

that these assumptions, as well as other possible orderings, resulted in system dynamics that were approximately equal in expectation to deterministic solutions of the continuous time disease model (Merl *et al.*, 2009, 2010).

3.2.1.2 Between-herd transmission

Between-herd transmission in the simulation model was explicitly driven by the movements of infected cattle between farms, which is dependent solely on the randomly generated contact network structure. All contact networks described in this section have been implemented in a freely available R package called **igraph** (Csardi and Nepusz, 2006).

In mathematical graph theory, the elements of a network are referred to as *nodes* or *vertices* (equivalent to farms), while the relationships between them are referred to as *edges* or *contacts*. Node *degree* measures how many direct contacts a farm has with others in the network. In directed networks, degree can be further partitioned into *in-degree* and *out-degree* representing the number of potential sources and sinks for disease transmission respectively. The degree of separation between any given pair of nodes in the network is measured by the *path length*. There are a number of theoretical networks formulated with specific structural composition to describe the distribution of nodes and the nature and extent of the contacts between them.

Here in the simulation model, a *random network* (Erdos and Renyi, 1960) was used to simulate the contact structure between farms. This assumes that N nodes were connected by E edges, which were chosen randomly from the set of $N * (N - 1)/2$ possible edges. Random networks are characterised by short paths and small degree of connectiveness, the distribution of node degree across the network is approximately *normal*. Within each simulation model run, the

`erdos.renyi.game` function in the `igraph` package was used to generate a contact structure with *random network* property.

In addition to the contact structure, the movement dynamics were also subject to stochasticity. Trade takes place each month and was simulated as a fixed rate across all farms in the network. Animals in each farm were randomly sampled according to this rate and the sampling results were aggregated to determine how many animals in each disease class departed (every animal have equal probability of been sampled for removal). The selling of animals is always directly to one other herd, which can be infected or uninfected. And the purchasing farm was chosen at random each month from a list of potential connections in the contact network.

Following from the assumption of constant herd size and immediate restocking of susceptible animals, only movement of infected animals were modelled. When infected animals were sampled for a proposed move onto another farm, it was assumed that susceptible animals of the seller herd increases and infected individuals decreases, and the opposite applies to the purchasing farm. If the randomly selected buyer has reached its maximum herd capacity (i.e. all animals in the herd were infected), the process was resampled from the remaining connected farms until a suitable farm can be selected. At the end of each movement activity, the losses and gains of animals from each disease class were updated accordingly.

The disease transmission dynamics within the theoretical contact network are depicted schematically in Figure 3.1.

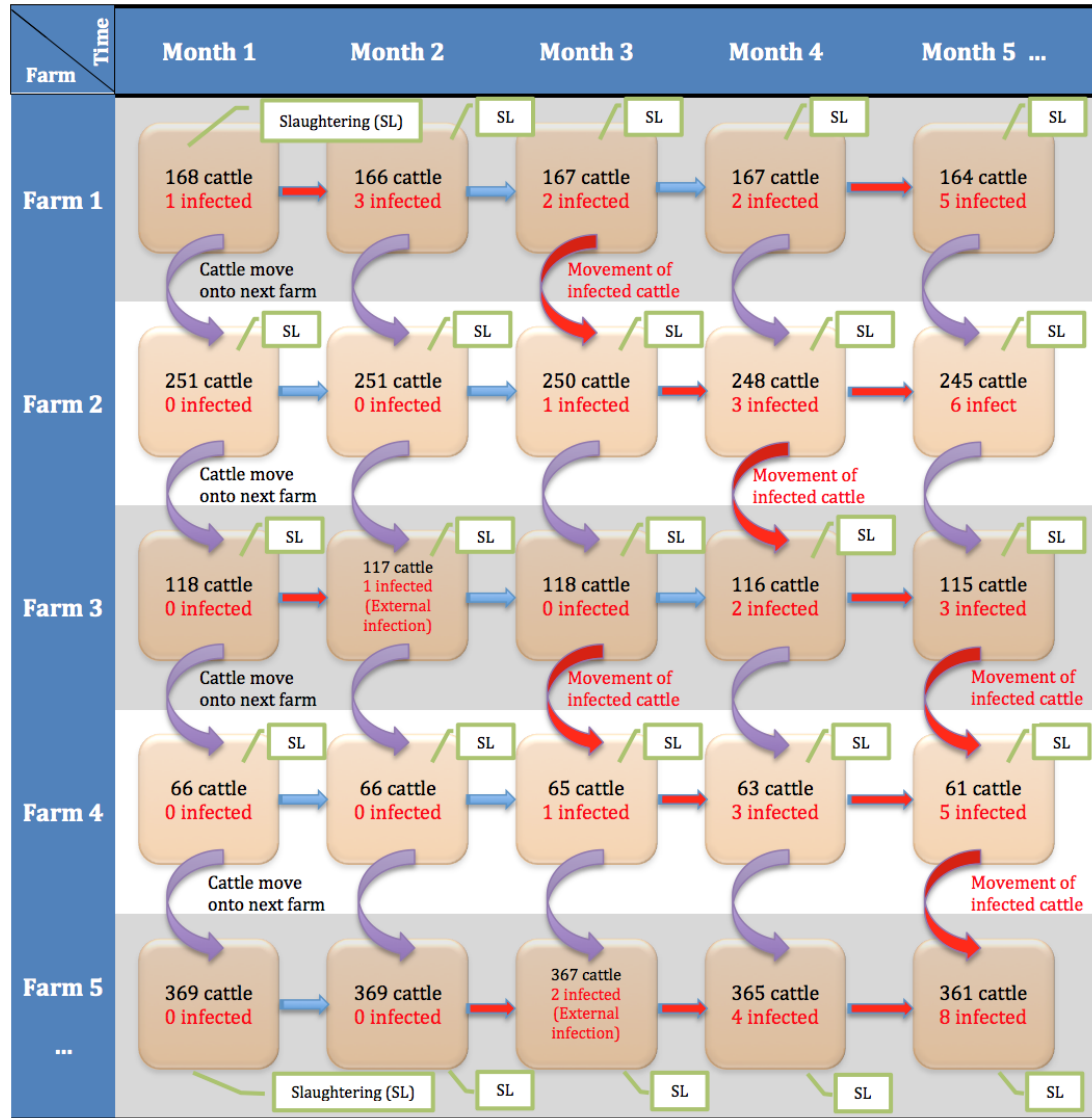


Figure 3.1: Disease propagation and movement dynamics between farms in the network on monthly basis. After initial introduction, disease spread either within-herd (horizontally) described by the SETI model (include external infection as described in section 3.2.1.1) on daily basis, or between herd (vertically), based the monthly movement rate and the underlying contact structure. Red arrows in the diagram (both horizontal and vertical) indicate a new infection has taken place.

3.2.1.3 Disease detection

The model accounts for two types of disease detection where infected animals can be identified and removed. These are regular monthly slaughterhouse meat inspection (SMI) and systematic routine whole herd test (WHT) using SICCT tests under standard interpretation. These surveillance tests are key to the current bTB control in agricultural stock in the UK, and therefore forms an important part of the simulation model.

In the SETI model structure, the exposed stage (E) was the first latent stage of the infection where the animal is infected but has not yet mounted a cell-mediated response to the invading *M. bovis* bacteria and therefore neither tests positive for the disease nor infects other cattle. The SICCT test is based on this response and so for the purpose of this model any test (including *post-mortem* SMI) on cattle in this stage will return negative. This also follows from common agreement that in non-infectious exposed animals, lesions are extremely unlikely to be found in the lungs and lymph nodes where animal carcasses are generally inspected (Fischer *et al.*, 2005). Current literature suggest between 0-65 days before infected cattle can mount an detectable immune response to the SICCT test (Kleeberg, 1960; de la Rua-Domenech, 2006; Thom *et al.*, 2006), and the total latency period (including the test sensitive stage) is approximately 150-265 days (O'Hare *et al.*, 2014; Biek *et al.*, 2012). However, in practice the duration of these states vary between cattle, and is dependent on the route of infection, herd-level demographics and other factors that may alter the immune response (Strain *et al.*, 2011). Therefore detection of a bTB breakdown herd is only established when one (or more) animals in the test sensitive and infectious stage have a positive test result. Thus, for each herd, the first positive case is the most important element in the detection process. After a herd was identified as infected, all infected individuals (including *exposed* and *test sensitives*) were

removed and replaced by susceptible cattle in order to keep the total herd size constant. In practice, the process of removing all infection on a particular herd may take a long time (Defra, 2010), since test positive herds are subject to several subsequent re-tests until it successfully passes two consecutive clear herd tests at intervals of at least 60 days apart (i.e. an indication that all infection was removed eventually). Detection of the infection was modelled by a probability of an animal being detected by each of the surveillance methods, where each surveillance has its specific test sensitivity. In reality, the test sensitivity can depend on the infection state of the animal, but for simplicity, the test sensitive and infectious stage were assumed to have the same level of sensitivity in the model. As the goal of this model was to simulate the propagation of infection, only the estimates of the test sensitivity (to determine the true positive test result) was needed, and not the test specificity, as false positive animals do not contribute to further infection (though specificity of SICCT are generally considered very good $\sim 99.8\%$).

The efficacy of the surveillance method was evaluated by calculating the herd-level test sensitivity (se_{herd}), which takes the form:

$$se_{herd} = 1 - (1 - Se)^d \quad (3.8)$$

This probability depends on the diagnostic test sensitivity (Se) and the absolute number of ‘infected and detectable individuals’ (d) tested on the occasion. At each test moment in time Eq. 3.8 was used in the simulations, reflecting the effect of test frequency. Once the herd-level detection probability was calculated on the specific test moment, a Bernoulli trial was conducted to determine the success of the test outcome. If the test was unsuccessful (i.e. missed), infection would continue to spread during the following time step.

The surveillance methods implemented in the model were applied to different pools of animals. The monthly SMI were performed on slaughtered animals in the abattoir, where each slaughtered infected animals (i.e. d in Eq. 3.8) was a detectable unit for the herd it came from directly before culling. The SICCT test was performed on all animals, where all animals in a particular herd were tested at the same time and each infected animal in the herd was a detectable unit. Eq. 3.8 can also be used when only part of the herd is SICCT tested, d being reduced by the fraction of the herd being tested, but this was not considered in the current model.

The SMI were performed each month and the culling rate was fixed for all herds, the routine SICCT test of a particular herd were implemented as discrete events on a pre-set point in time. Unless stated otherwise, before starting each simulation, all herds were assigned a random start date within a certain period of time for which to begin testing.

The disease detection process is shown schematically using a flow-chart in Figure 3.2

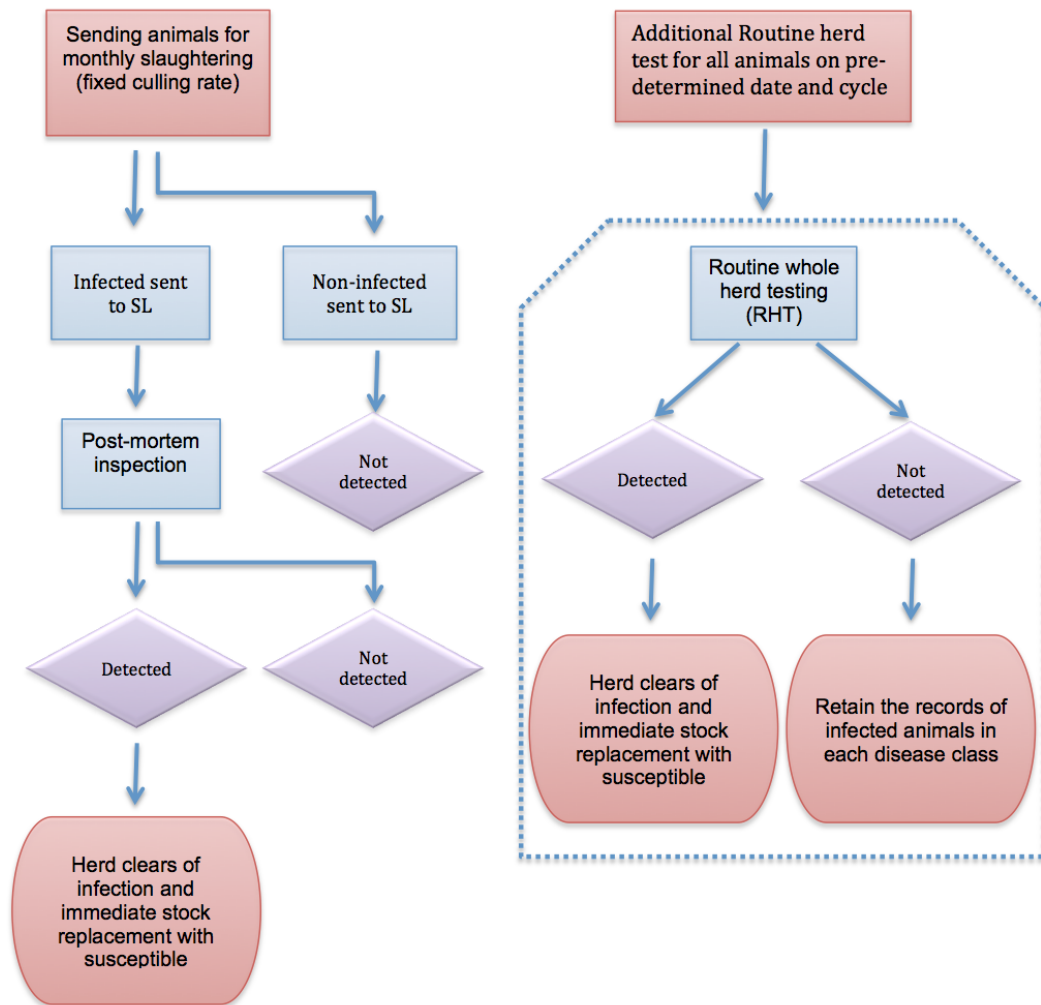


Figure 3.2: Disease detection mechanism that includes regular monthly slaughter and routine herd tests. Animals sent to slaughter were randomly sampled from the herd according to the fixed monthly culling rate, and routine herd tests were conducted on whole herd basis.

3.2.2 Model initialisation and parameter values

In the simulation model, disease outbreaks were initially seeded by introduction of a single infectious animal on a randomly chosen herd. Each simulation represents a simulated outbreak of bTB in a randomly generated contact network, and was ended at the pre-set end time of 15,000 days after the initial introduction of infection. The model was operated with a time step of 1 day and, except where specifically noted, all model parameters and their initial default values were defined and summarised in Table 3.1.

Individual herd profiles were mapped based on 543 herds from Wigtown county (only farms with at least one previously recorded RHT) situated in south-west of Scotland, the herd sizes were fixed according to their average annual herd size from 2002 to 2012. This information was extracted and calculated from the CTS “farm population” table and ranges from 1 to 4784 animals per herd. The mean herd size across all farms in the simulation model was 258 (median: 284). Furthermore, the population dynamics in the herd are subject to management practice from farmers. The decision rules accounted for in the stochastic model involved culling and trading. In order for the model to simulate realistic population dynamics with average culling and trading rates according to practice, data extract from CTS animal movement records from 2002-2012 in Wigtownshire were used to calculate the parameter values. The default monthly culling rate was fixed across all farms and is calculated as $1/\text{mean age at death}$ (in month) for all animals identified on farm. Similarly, monthly trading rate for each herd was also fixed and is given by $\text{mean number of movements}/\text{mean age at death}$ (in months). Following this method of calculation, the estimated monthly culling and trading rates were 5% and 6% respectively (of the total herd size). Moreover, according to the movement data, herd trade with 6 different herds on average per year, therefore the average connection parameter when generating the

contact network was fixed at 6 for each herd (Table 3.1). Animals for both culling and trading were chosen at random each month as a fraction of the total herd size which can be in any disease classes. Finally, previous RHT records (with test dates) from the 543 herds were also extracted from the Sam's IT system, and was applied in the simulation model to form a basis for comparison between alternative surveillance strategies.

Within-herd transmission coefficient β in the model is defined as the probability that a new infection occurred per infectious animal per day. This quantity was estimated using the average within-herd incidence rate from the empirical data analysis in Chapter 2 where the study was conducted on Scottish cattle herds that were confirmed with infection. The results demonstrated variations in the rate between farms due to herd sizes and the length of disease duration (i.e. from initial introduction to removal of last infection), the overall average incidence rate (from 97 farms (refer to chapter 2)) of 0.00006 per infectious animal per day was used as an estimate for the transmission coefficient β in the simulation model. From the mass action model βSI (Stegeman *et al.*, 2002), this implies that on average, an infectious animal will cause 2.31 new infection per year in a herd with 100 animals. This estimate is similar to (Barlow *et al.*, 1997) and also falls within the range in previously published results from Brooks-Pollock *et al.* (2014) and O'Hare *et al.* (2014).

Transition probabilities between the other states were density independent and equal (fixed) for all infected animals. The values for the transition probabilities σ and γ were chosen on the basis of existing field and experimental estimates which was obtained based on estimates of the average duration of each latent state. The rate that exposed cattle becoming test-sensitive, and test-sensitive cattle becoming infectious is the inverse of the exposed and test-sensitive periods

respectively (O'Hare *et al.*, 2014). Data on the exposed state E from previous animal challenge studies were observed to lie between 0-65 days (Barlow *et al.*, 1997; Kleeberg, 1960; Dean *et al.*, 2005; Thom *et al.*, 2006), however, when considering multi-herd transmission and depending on local disease prevalence, the estimated range was between 0-119 days (Conlan *et al.*, 2012; O'Hare *et al.*, 2014). A larger estimate of 100 days were used for σ due to the assumption that the time to infectiousness is likely to be longer when resident animal is infected from external sources in comparison to introduction of disease from already infected animal. The former may dominate in high risk areas, and the latter would usually be the case in low risk areas (Green *et al.*, 2008). In contrast, the duration of the test sensitive state T shows much more variation, with a minimum of 7 weeks in an infection experiment with calves (Neill *et al.*, 1991) to a maximum of 80 weeks (upper estimate of Livingston in Barlow *et al.* (1997)). The estimate of O'Hare *et al.* (2014) of 200 days were used as an estimate to derive γ , this also agrees with previously published estimates of 180 days \pm 20 days from Conlan *et al.* (2012) and Biek *et al.* (2012) (see Table 3.1; parameter σ and γ).

The estimate for the external force of infection α of 5×10^{-7} was also derived from O'Hare *et al.* (2014). Under the conventional approach, the external routes of transmission were modelled through a single generalised infectious pressure that incorporates all external sources of infection such as contiguous spread from neighbouring herds, the presence of a wildlife reservoir and infection introduced from inward cattle movements. However, as described in section 3.2.1.2, the simulation model already accounts for disease introduction from local animal movements (within the contact network), the parameter estimate therefore was adjusted to remove the effect of this external factor, therefore an estimate of 1×10^{-7} was used in the simulation model run. (see Table 3.1; parameter α).

Estimates for the SICCT and SMI test sensitivities were taken from a meta-analysis performed by the Veterinary Laboratories Agency (AHVLA) (Downs *et al.*, 2011) and are shown in Table 3.1. Although fixed estimates were used for test sensitivities in the simulation model, parameter values can be easily adjusted to account for various different tests with different levels of interpretation (by adjusting individual level sensitivity). The tests were assumed to be conducted independently in the model, this means that when multiple tests were due on the same day, detection process was repeated for the individual test occasion, therefore the conventional “system test sensitivity” for combined tests does not need to be calculated.

Table 3.1: Summary of model parameters and their initial input values used in the stochastic simulation runs

Parameter name	Description	Default value in model	Range of values for the sensitivity analysis	Source of default parameter estimate
t	Time step (Δt)	1 day	—	—
Within-herd transmission				
α	External force of infection	1^{-7}	0, 1^{-8} , 1^{-6}	Adjusted from O'Hare <i>et al.</i> (2014)
β	Within-herd transmission coefficient	6^{-5}	$[1^{-6}, 1^{-2}]$	Results from Chapter 2
σ	Transition probability E \rightarrow T	0.01	—	(O'Hare <i>et al.</i> , 2014)
γ	Transition probability T \rightarrow I	0.005	—	(O'Hare <i>et al.</i> , 2014)
Between herd transmission				
N	Number of farms (Nodes)	543	—	CTS data of Wigtown county
E	Average connections per farm (Edges)	6	—	(Kiss <i>et al.</i> , 2006a)
M	Average monthly movement rate (trade)	6%	—	Calculated from CTS movement data
Disease detection				
C	Average monthly culling rate	5%	—	Calculated from CTS
se_{SICCT}	SICCT test sensitivity	0.50	0.70, 0.90	(Downs <i>et al.</i> , 2011)
se_{SLH}	Slaughterhouse test sensitivity	0.70	0.50, 0.90	(Clegg <i>et al.</i> , 2011a)

3.2.3 Model implementation

A number of surveillance options were explored to evaluate the impact on the simulated bTB epidemic based the intensity and frequencies of routine herd testing. Specifically, the following surveillance scenarios were modelled in combination with the regular monthly SMI:

- Farms were tested based on the previous RHT test dates between 2002 - 2012 from the Sam's IT system.
- All farms were tested at the same time under 4 year RHT.
- All farms were tested within 6-months with random start dates and every 4 years thereafter.
- All farms were tested within 1-year with random start dates and every 4 years thereafter.
- All farms were tested under 4 year RHT with evenly distributed start dates
- SMI tests only (i.e. no RHTs).
- 6 year RHT with random start dates for all farms.
- 4 year RHT with random start dates for all farms.
- 2 year RHT with random start dates for all farms.
- Annual routine herd tests with random start dates.

3.2.4 Model sensitivity analysis

Previous analysis has shown that the transmission coefficient β and the individual-level test sensitivity for each diagnostic test (i.e. se_{SICCT} and se_{SMI}) are significant factors that can effect the simulation model outcome (Barlow *et al.*, 1997; Fischer *et al.*, 2005; Brooks-Pollock *et al.*, 2014). Different values for these parameters were applied to the same surveillance strategy (other components in the model remaining fixed) to elucidate the impact on the disease spread and detection in the simulation model. In addition, in low risk and low prevalence areas where evidence of established wildlife reservoir is less apparent and probability of disease introduction from animal exports are substantially less compared to high risk areas, the underlying external factor of transmission would be comparatively small. Similarly, high risk areas have higher external force of infection. Modelling disease spread in either low or high prevalence areas (with different levels of background risk of disease introduction) were characterised by altering the rate of external force of infection, α . Thus, during the sensitivity analysis, while maintaining the same routine surveillance strategies, the relative influence of varying these epidemiological parameters on the patterns of bTB spread were also explored. Three separate epidemiological scenarios were analysed based on different levels of (1) within-herd transmission (β), (2) external force of infection (α) and (3) individual-level test sensitivities (se_{SICCT} and se_{SMI}). Table 3.1 shows the prescribed range of values used in the simulation models.

Moreover, it was acknowledged that the types of contact structure may also impact disease spread, particularly in diseases where animal movements can potentially contribute to significant proportions of infection (Dube *et al.*, 2009; Martinez-Lopez *et al.*, 2009). Therefore, different types of contact network were analysed to explore changes in disease transmission pattern and time of detection. Specifically, in addition to the random contact network structure, scale

free contact structures were also used to simulate between-herd transmission. Early work on human sexual contact networks first established that a small number of individuals with atypically high rate of contact were disproportionately responsible for the epidemic spread of HIV as well as other sexually transmitted diseases (Gupta *et al.*, 1989). Subsequently, this principle has been referred to as the '20/80' rule, which reflect the common findings across many biological systems that 20% of the host population often contributes to 80% of the transmission potential for infectious pathogens (Woolhouse *et al.*, 1997). Cattle movements are no exception to this rule (Woolhouse *et al.*, 2005). The highly right skewed or power-law degree distribution in these networks lead to the emergence of *scale-free* behaviour as described by Barabasi and Albert (1999). This behaviour is characterised by the absence of epidemic thresholds in large populations (Barabasi, 2009), higher basic reproduction numbers (R_0) than expected for network with uniform degree distributions (Woolhouse *et al.*, 2005), and greater tolerance to control measures applied to the network at random (Albert *et al.*, 2000). In the simulation model, a scale-free network was also used to simulate the disease spread with 4-year RHT to evaluate the bTB epidemic in comparison to the random networks. The contact structure was generated using *barabasi.game* function under the *igraph* package in R.

500 simulations were conducted under each simulation scenario and the mean summary statistic was used to compare the size and patterns of disease spread. Each simulation scenarios were evaluated by comparing the total number of infected animals and herds in the system. The initial time point of disease detection were also calculated to assess the response of the surveillance strategies under each analysis. The simulations were run in a graphics processing unit (GPU) based parallel environment where each simulation was submitted and run in parallel before results were compiled and summarised in R (R, 2012).

3.2.5 Model assumptions and simplifications

The following assumptions were made in the model formulation.

- The model assumes that when infected farms were detected by routine surveillance (i.e. either through regular slaughter or RHT), all infected animals (i.e. exposed, test sensitive and infectious) on the farm were subsequently detected and the herd immediately restocks to its original size in the next time step (i.e the herd becomes healthy and is immediately susceptible to new infection on the following day).
- The model assumes constant monthly movement rate and slaughter rate for each farm (the number of animal movements and slaughter depends on the herd size and was fixed throughout the simulation).
- In each simulated epidemic, constant herd size was assumed where animals removed due to natural death or outward movement were immediately replaced by susceptible animals (e.g. births). The effect of having some infectious replacements was subsumed in the external force of infection term, αS .
- Also assumes constant number of herds (i.e. no introduction of new herds or removal of old herds).
- There is no distinct profile for each animals, except which states they belong to. Hence, there are no age structures for individual animals in the model.
- The βSI mass action function adapted for the transmission function assumes homogenous mixing between infected and susceptible individuals, and contacts between them were random within a herd of fixed size.

- Simplifying assumption of homogenous susceptibility and infectiousness. Potential individual heterogeneity in “super-spreaders” and “super-susceptible” were not incorporated.
- Both the SICCT test and slaughterhouse testing were assumed to have fixed level of sensitivity while specificity is assumed to be 100% in the current model structure (existence of anergic animals were not considered).
- No age discrimination between individual animals (i.e. the model does not incorporate age structure).
- Only whole herd tests were considered for RHT, this assumes every animal were subject to the test
- Transition probabilities between the disease states in the SETI model structure are density independent and equal (fixed) for all infected animals.
- Only routine surveillance methods (i.e. WHT and SMI) were considered in the current model formulation, other types of control measures such as contact tracing, contiguous herd test and pre- and post-movement tests were not incorporated.
- The effect of movement restriction as a consequence of herd breakdowns and subsequent follow up testings and tracing tests were not considered.

3.3 Results

Based on previously recorded RHT test dates (obtained from the Sam's IT system), the 543 herds used for the simulation analysis had a total of 1,723 unique routine herd tests between 2002 - 2012. The average number of RHT per year was 157 with the highest total number of RHT recorded in 2002 (227 tests) and lowest in 2009 (89 tests). The monthly distribution of RHT count displayed in Figure 3.3 shows a seasonal variation in routine testing with most tests conducted at the beginning or end of the calendar year, and the fewest conducted during summer months between June-September. Due to the nature of RHT in low incidence areas (i.e. tested on 4 yearly basis), the testing pattern also repeats itself on a 4-yearly cycle.

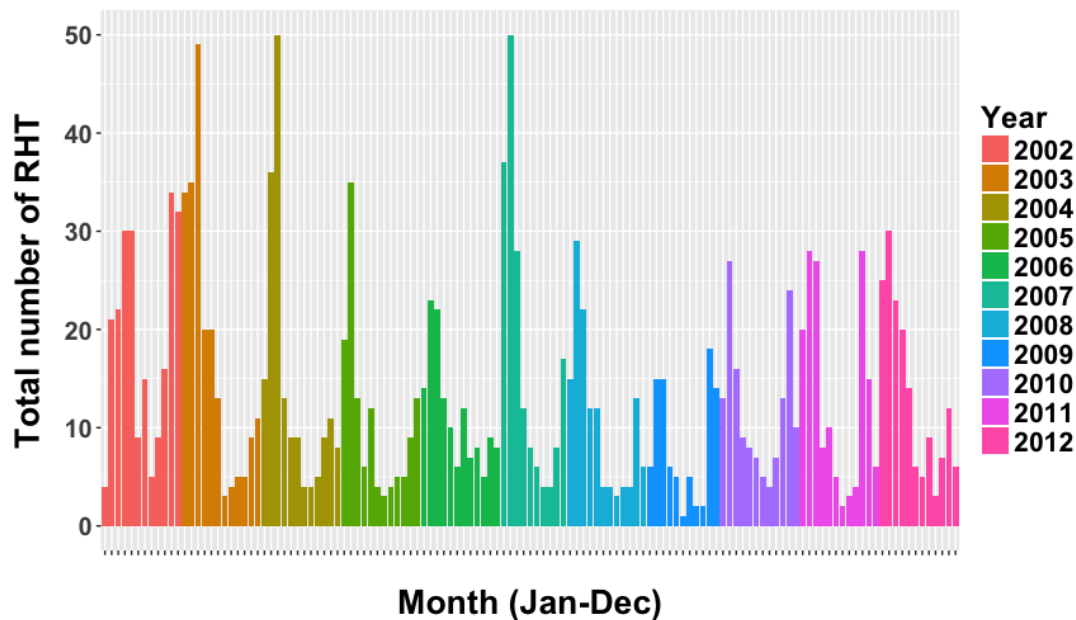


Figure 3.3: Monthly aggregated RHT count for 543 farms in Wigtown county of Scotland between 2002 - 2012. Each bar represent a particular month of the year, with order from left to right representing January - December

The results from simulated bTB epidemics, using the default parameter values in Table 3.1 with historical RHT test dates, are represented in Figure 3.4. With the combination of monthly routine slaughterhouse meat inspection, and historical 4-year RHT, the disease starts off slowly from a single infectious case and spreads through the farm network overtime following the process of within-herd and between herd transmission described in Section 3.2.1.1 and 3.2.1.2.

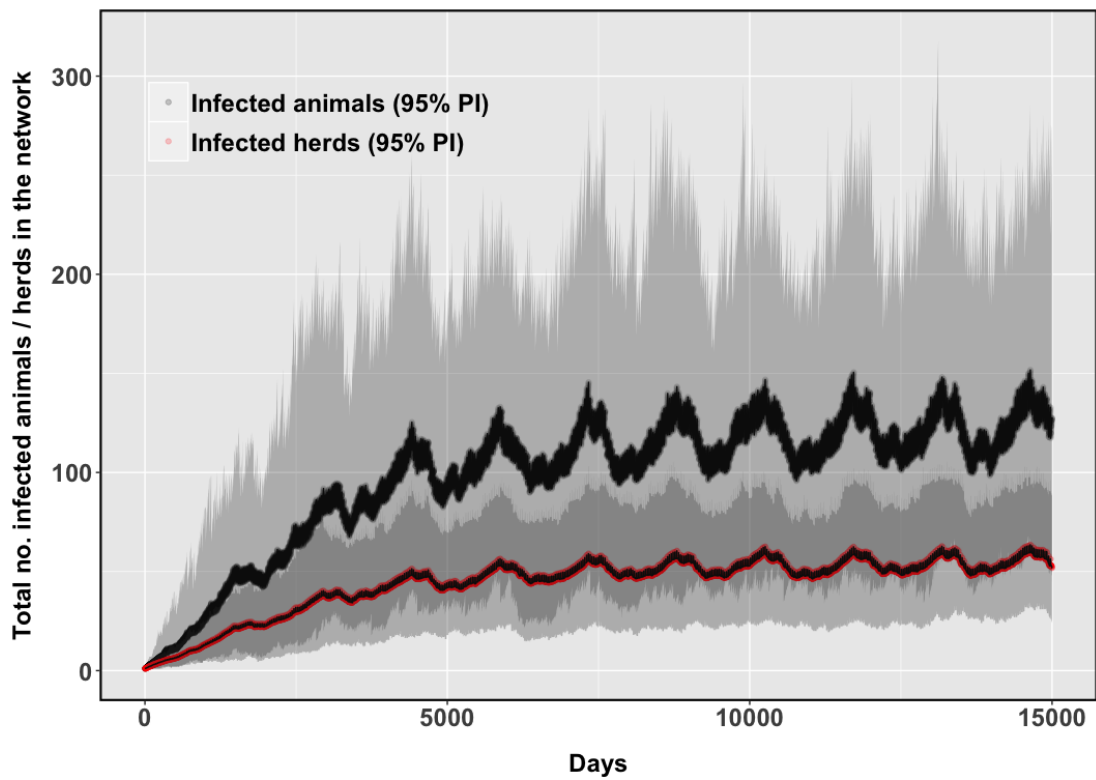


Figure 3.4: The simulated emergent dynamics of bTB infection in a small population of 543 farms, using the SETI stochastic model of infection with 2002 - 2012 RHT dates obtained from Sam’s IT system. The trend represents the mean number of infected animals in the network across 500 simulations, and the 95% empirical prediction interval is displayed in shaded regions

Based on 500 simulations of disease spread under this scenario, the first detection occurred on 243 days (mean estimate) after the initial infection. In

addition, at the time of initial detection, there were on average, 12 infected animals (5 in exposed stage, 4 in test sensitive and 3 in infectious) in the detected herd. The total number of infected animals in the network reaches a local asymptotic maximum (125 infected cases out of approximately 140,000 animals) around 4,400 days after initial infection (mean from 500 simulation runs). Similar to the monthly RHT count (Figure 3.3), the total number of infections also shows a 4-year cyclic pattern (driven by the testing pattern). From the simulated epidemics, the long term trend shows that the disease eventually converges to a steady state with infection number oscillating between 86 (trough) and 152 (peak) in a 4-yearly cycle. Similar trends were also observed in the total number of infected herds, where after 5,000 days, the number of infected farms (nodes) in the network fluctuate between 41 and 64 for every 4-years. In comparison to the simulation under existing RHT dates, the model was run using random start dates for routine herd testing and repeat every 4-years thereafter. The pattern of the overall epidemic size (in terms of total infected animals) also shows a steady increase before settling into a stable level (Figure 3.5). However, the fluctuation between different years were more consistent and over the 500 simulated disease episodes, the mean global asymptotic maximum of infected cases was 140. This is marginally smaller in comparison to results from Figure 3.4 (152 cases). The maximum number of infected farms (59) also showed a small reduction compared with results using historic testing dates (64). Under random testing dates, the initial detection occurs 261 days (mean from 500 simulation runs) after disease introduction, and there were 12 infected animals observed at that point (same result as testing under historical RHT dates).

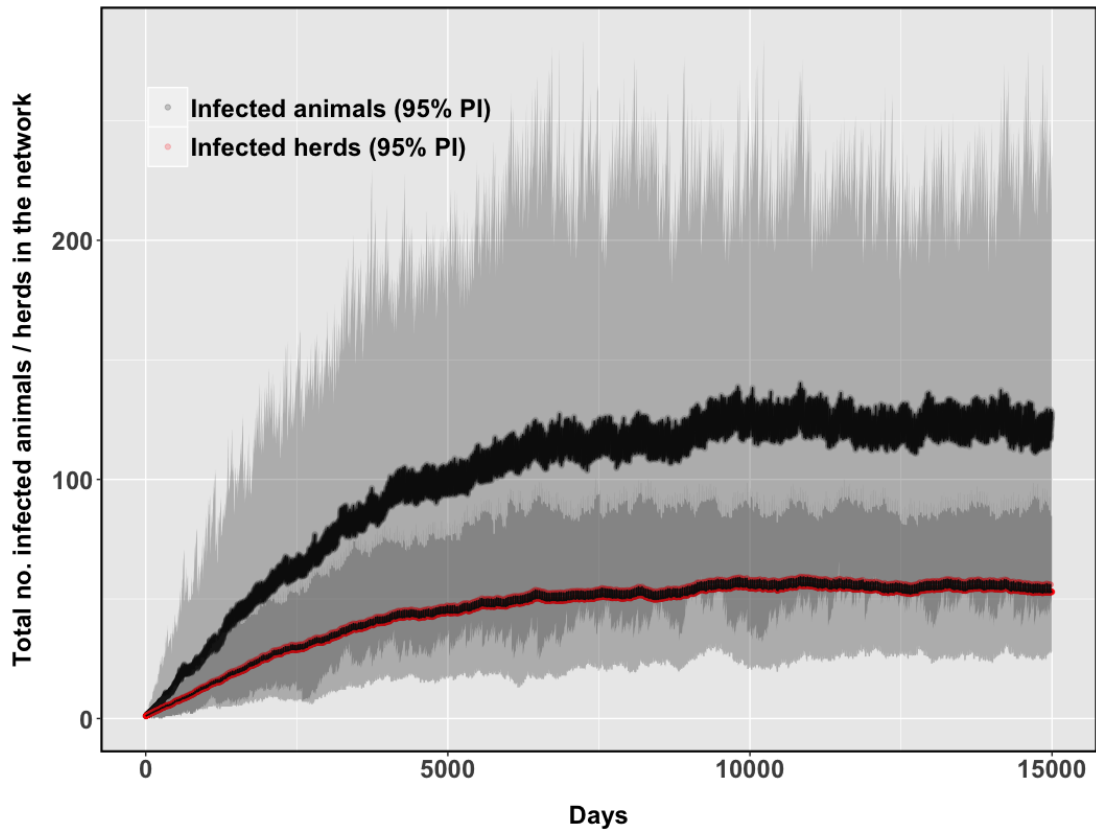


Figure 3.5: The simulated emergent dynamics of bTB infection in a small population of 543 farms, using the SETI stochastic model of infection with random start dates for RHT, and repeated every 4 years. The trend represents the mean number of infected animals in the network across 500 simulations, and the 95% empirical prediction interval is displayed in shaded regions

In contrast, rather than using 4-year RHT on random occasions (i.e. completely random starting point to initiate routine herd testing), Figure 3.6 shows variation in simulated epidemics under different testing patterns (i.e. varying the intensity of testing within a particular time frame) while keeping the disease transmission process constant. In particular, the 4-yearly RHT was pre-scheduled to be (1) evenly spread across time, so that approximately 11 farms were tested each month (Figure 3.6a); (2) within 1 year, where all farms were tested within 365 days (Figure 3.6b); (3) within 6-month (Figure 3.6c); and (4) on the same day (Figure 3.6d). The frequency of the routine surveillance does not change, and therefore the total number of tests remains to be the same across the time period. In analysing results from disease simulations under different routine herd tests, the daily maximum number of infections observed (both in terms of infected animals, and infected herds) were used as indicator to the epidemic size, since in the long run, the disease slowly converges to this point and maintains at this level (as shown in Figure 3.6).

For increased intensity of testing within a shorter time frame, the detection of infected cases increased significantly during the testing window (larger reduction in infected animals as farms were tested more closely together, Figure 3.6), however, this also resulted in a faster build up of infection for periods outside of testing. Under all scenarios, the disease reached a stable level, though overall there was a relative small percentage change in the maximum number of infection across the 4 testing strategies (Table 3.2). The initial time of disease detection was generally sooner when RHT were conducted closer together in comparison to random or evenly distributed testing dates and the infected cases at the time of detection were also lower.

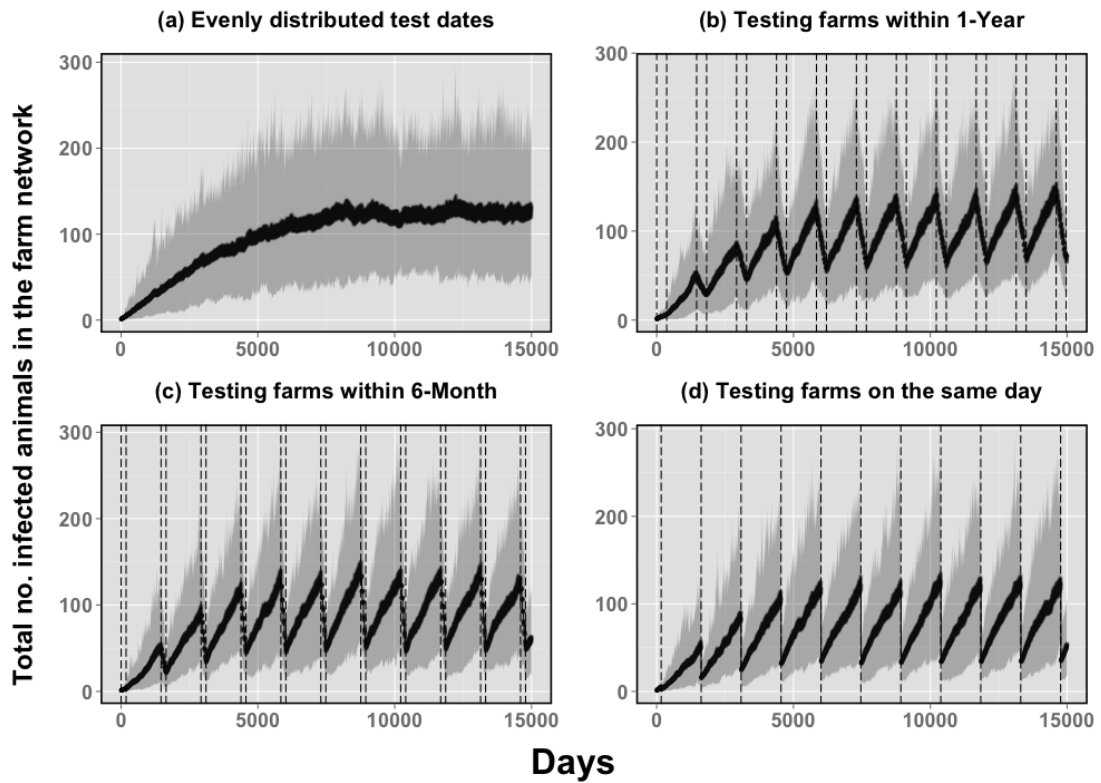


Figure 3.6: The simulated dynamics of bTB infection in 543 farms, using the SETI stochastic model of infection with (a) evenly distributed start dates for RHT across all farms; (b) RHT testing clustered within a year; (c) RHT testing conducted within 6-month; (d) all farms were tested under RHT on exactly the same day. The trend represents the mean number of infected animals in the network across 500 simulations, and the 95% empirical prediction interval is displayed in shaded regions

Table 3.2: Mean summary statistics from 500 simulation model runs under different routine surveillance strategies with random start dates. The “Max no.” refers to the expected maximum number from 500 simulation model runs (i.e. the mean(max)).

Simulation scenario	Max no. of infected animals (at any time)	Max no. of infected farms (at any time)	Time of Initial detection in days (infected animals in detected herd)
RHT with evenly distributed dates	145	59	246 (10)
RHT within 1-year	154	66	185 (6)
RHT within 6-month	151	62	158 (7)
RHT on same day	135	57	189 (7)
Slaughterhouse alone	456	149	271 (12)
6-year RHT	206	82	262 (11)
4-year RHT	140	59	261 (12)
2-year RHT	49	22	244 (12)
Annual RHT	20	10	220 (8)

As well as varying test intensity, different frequencies of routine herd test (using random start dates) were also implemented to observe the impact on the overall epidemic (Figure 3.7). As with previous simulations, the transmission process and herd dynamics were kept consistent across different testing strategies. The mean number of infected animals from 500 simulations indicate that with increased routine herd tests (i.e. more surveillance effort) the overall epidemic size in the long run was substantially reduced. However, based on slaughterhouse surveillance alone, the epidemic shows an increasing trend without signs of reaching its asymptotic maximum within the pre-set time period (15,000 days). With the addition of regular routine herd tests, the disease was able to be maintained at a constant level (i.e. non-increasing) in the long term. In particular, compared to slaughterhouse surveillance alone, the addition of 6-year and 4-year RHT offered a 55% and 70% reduction in the maximum number of infected animals respectively. While 2-year and annual RHT delivers a further 20% - 25% reduction in the number of infections in relation to the slaughterhouse only scenario (Table 3.2). Similar percentage drops were also achieved for the number of infected farms amongst different testing strategies, however, the increase in the speed of initial detection appeared to be less dramatic as the frequencies of RHT was increased.

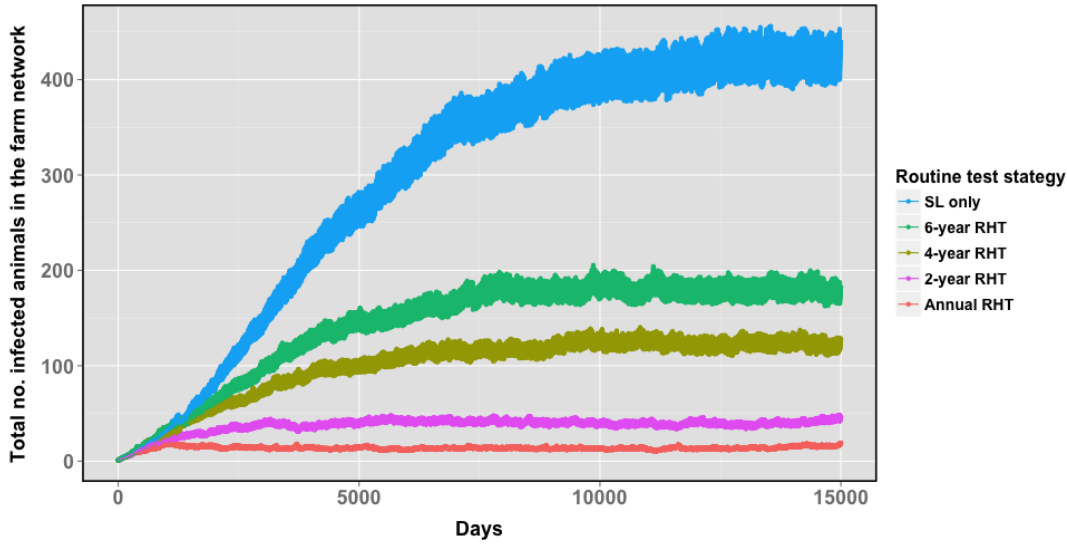


Figure 3.7: The simulated dynamics of bTB infection in 543 farms, using the SETI stochastic model of infection with different routine testing frequency. The trend represents the mean number of infected animals in the network across 500 simulations.

3.3.1 Model sensitivity analysis

The current models use the same transmission parameters to evaluate the difference in the simulated epidemic for different surveillance strategies. From the results in Chapter 2, the within-herd transmission rate (β) is likely to vary with different farms, and can be affected by factors such as herd size, area based risk of infection and herd management practices. Accordingly, to check the results for sensitivity to the within-herd transmission rate, the simulation model was re-evaluated with different levels of within-herd transmission rate (i.e. with different β values in the SETI structure), while using the same routine testing strategy (i.e. 4-year RHT with random start dates) and maintaining other model components the same. The results from the disease simulation varied substantially when β was decreased by a factor of 10 (Figure 3.8). Although under all scenarios, the pattern of the epidemic eventually reaches a constant level and maintains at that

point. When β was changed from 6^{-3} to 6^{-4} , the maximum number of infections (mean from 100 simulations) was reduced by more than 75% (from 9000 infected cases to 2194). When β was further reduced to 6^{-5} (i.e. default value used for the main simulation models), the maximum infected animals was 140, this represents a 94% reduction compared with the previous level (Table 3.3). As β falls to 6^{-6} , the maximum number of infection was only 14 cases across the entire duration of infection.

The reduction in the maximum number of infected farms was not as dramatic as β was reduced from 6^{-3} (328 infected farms) to 6^{-4} (271 infected farms), however, when β was reduced below 6^{-4} , there was a substantial drop in the number of infected farms (i.e. from 271 to 59 infected farms). Moreover, the inverse relationship was observed for the time to initial detection. As a consequence to the increased transmission rate, initial disease detection tends to happen a lot sooner as infection within the network builds up at a much faster rate (see top half of Table 3.3).

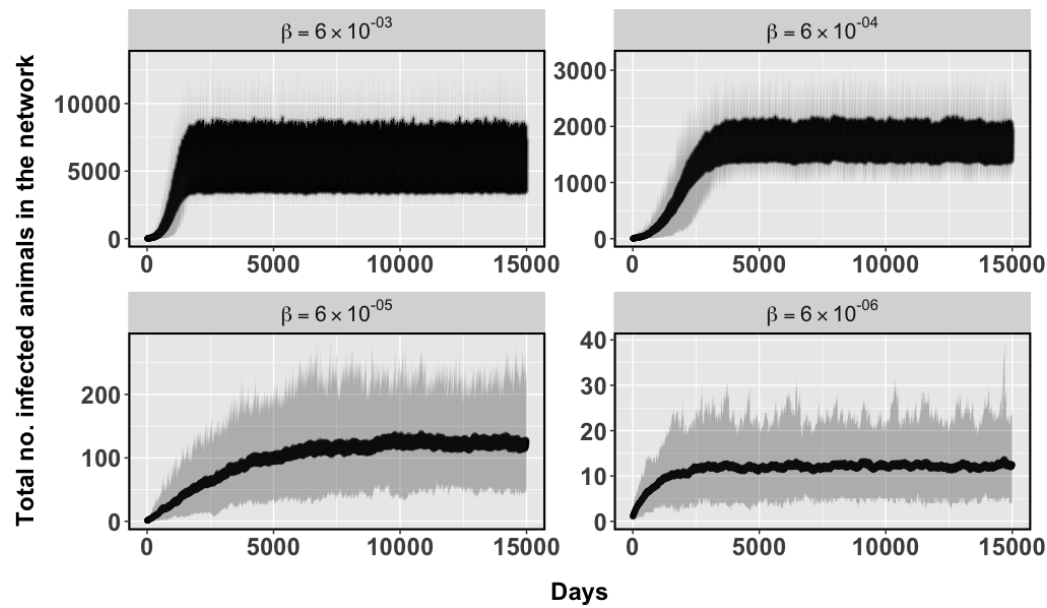


Figure 3.8: The simulated dynamics of bTB infection in 543 farms, using the SETI stochastic model of infection with different within-herd transmission parameter while maintaining all other factors to be fixed at default value. Routine herd test were conducted on 4-yearly basis with random start date. The trend represents the mean number of infected animals in the network over 500 simulations, and the 95% empirical prediction interval is displayed in shaded regions.

Table 3.3: Mean summary statistics from 500 simulation model runs under 4-year RHT strategies with random start dates, while altering the values of within-herd (β) and external transmission parameters (α) in the SETI model structure. The “Max no.” refers to the expected maximum number from 500 simulation model runs (i.e. the mean(max)). While altering β , the default value of $\alpha = 10^{-7}$ was used (highlighted in bold), and while altering α , the default value of $\beta = 6^{-5}$ was used (highlighted in bold).

Simulation scenario	Max no. of infected animals	Max no. of infected farms	Time of initial detection in days (infected animals in detected herd)
$\beta = 6^{-3}$	9000	328	79 (114)
$\beta = 6^{-4}$	2194	271	142 (37)
$\beta = 6^{-5}$	140	59	261 (12)
$\beta = 6^{-6}$	14	11	290 (5)
$\alpha = 10^{-5}$	1044	330	60 (82)
$\alpha = 10^{-7}$	140	59	261 (12)
$\alpha = 10^{-9}$	7	3	692 (7)
$\alpha = 10^{-11}$	6	2	381 (7)
$\alpha = 0$	4	2	337 (5)

The external force of infection is a major component in the epidemic model that represents external disease introduction from outside of the farm network, it can have a significant effect on the transmission process. Therefore the simulation models were also tested with different levels of external force of infection (i.e. α values within the SETI model structure). The results of this analysis were summarised in Figure 3.9 and bottom half of Table 3.3. When external infection was below 10^{-9} or completely eliminated from the disease transmission process (i.e. α was set to 0), the maximum infection within the network was relatively low, with between 4-7 cases from 2 or 3 infected farms (Table 3.3). While for an external force of infection that exceeds 10^{-7} , the maximum infected cases can be as high as 1,044 with more than 60% of farms infected in the network. Similar to the within-herd transmission parameter, the initial disease detection occurs at much quicker rate with higher levels of external infection, however, as the rate of external force of infection becomes so small (lower than 10^{-9}), there were very low levels of infection in the network at any given time. This has resulted in delays for disease detection as shown by bottom two lines of the results in table 3.3 compared to more prevalent scenarios.

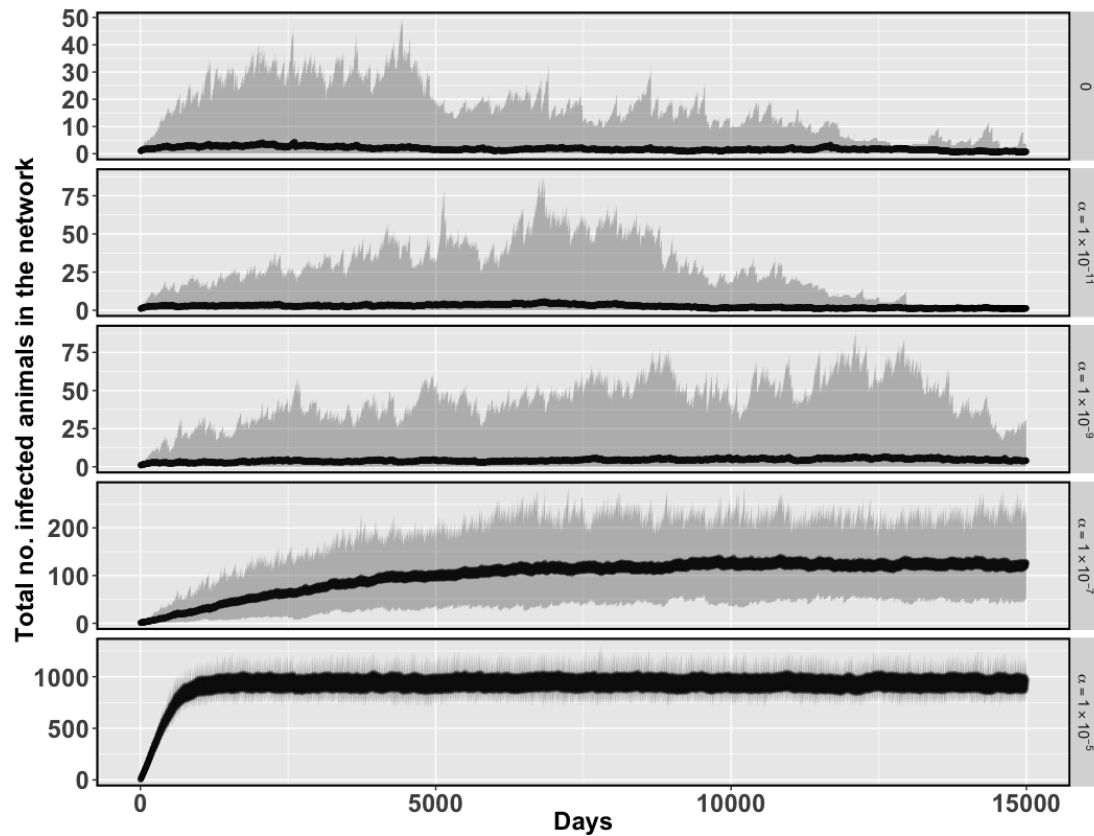


Figure 3.9: The simulated dynamics of bTB infection in 543 farms, using the SETI stochastic model of infection with different rates of external force of infection while maintaining all other factors to be fixed at default value (see Table 3.1). Routine herd test were conducted on 4-yearly basis with random start dates. The trend represents the mean number of infected animals in the network at any given time across 500 simulations, and the 95% empirical prediction interval is displayed in shaded regions.

The ability for any surveillance activity to identify infected individuals is directly related to the individual-level sensitivity of the diagnostic test employed. The two tests that were used in the simulation models were regular slaughterhouse meat inspection (SMI) and routine whole herd tests on all animals present on the farm using SICCT. The simulation models were analysed with different combinations of SICCT and SMI sensitivity from a range of values between 30% - 90%. The underlying results from 100 simulations were summarised as a matrix plot in Figure 3.10. On average, for every 20% increase in SMI sensitivity, the maximum number of infections in the network was reduced by 60%. On the other hand, for every 20% increase in SICCT sensitivity, the maximum infection were reduced by approximately 20%. Unsurprisingly, the minimal infection occurs when both SMI and SICCT sensitivity were at 90%, this corresponds to a maximum of 46 infected animals in the network (bottom right plot in Figure 3.10). In contrast, simulation results with SMI and SICCT sensitivity of 30% produced a maximum of 1,261 infected cases (top left plot in Figure 3.10).

As well as analysing the variations in within-herd transmission processes which were governed by the internal transmission parameters in the SETI model structure, the between-herd transmissions were described by the types of contact network structure in the model formulation. For this part of the analysis, a scale-free type contact network was compared with a random contact structure. The results suggest that the overall epidemic pattern settles into a steady state much faster (around 1,000 days after model initiation) with low levels of infection under the scale-free network structure (Figure 3.11). In particular, it offers more than 75% reduction in infected cases (32 maximum infected cases) with faster initial disease detection in comparison to a random contact network (140 infected cases). Similar results were also true for the number of infected farms, where scale-free network had a maximum of 11 infected farms compared to 59 under the random contact structure.

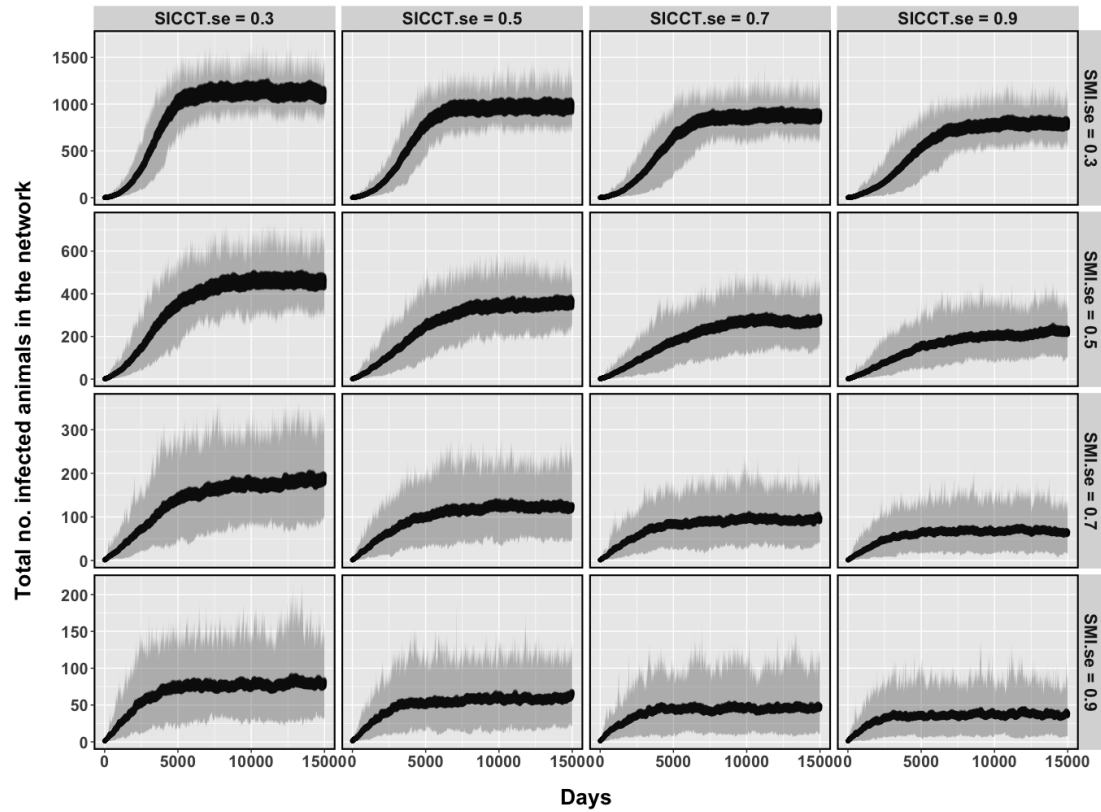


Figure 3.10: The simulated dynamics of bTB infection in 543 farms, using the SETI stochastic model of infection with different combinations of SICCT and SMI sensitivity while maintaining all other factors to be fixed at default value (see Table 3.1). Routine herd tests were conducted on a 4-yearly basis with random start dates. The trend represents the mean number of infected animals in the network across 500 simulations, and the 95% empirical prediction interval is displayed in shaded regions.

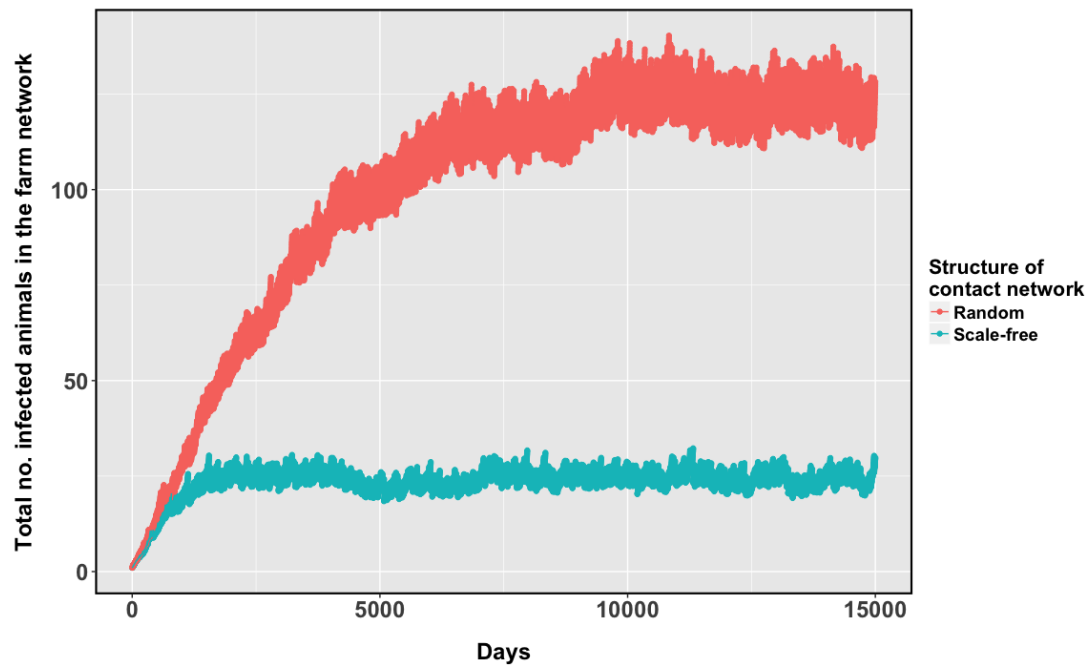


Figure 3.11: The simulated dynamics of bTB infection in 543 farms, using the SETI stochastic model of infection with random and scale-free contact structures to model between herd transmission, while maintaining all other factors to be fixed at the default value (see Table 3.1). Routine herd test were conducted on a 4-yearly basis with random start dates. The trend represent the mean number of infected animals in the network across 500 simulations.

3.4 Discussion

This chapter has described the development of a stochastic individual (premises)-based simulation model to evaluate different routine herd testing strategies on the spread of bTB. The model was developed to simulate a chain of infected herds, where each herd was followed in time. Different surveillance strategies were applied to mimic the disease situation for a small network of farms after the introduction of one infected animal into one herd. Individual herd profiles such as herd size, previous RHT history, average monthly slaughter rate and movement rate were taken from 543 farms in Wigtown county in Scotland.

Results from historical herd testing recorded in the Sam's IT system show a seasonal variation for routine herd tests amongst these farms. Most RHT (more than 60%) were conducted at the beginning or end of the calendar year during the winter months (between November - March), where animals are likely to be housed together compared with when they are grazing outdoors in summer months. This is simply because it is easier to gather and test the cattle when they are already contained within a building and therefore offers logistic advantage in performing a whole herd test (Defra, 2015b). Comparison between the simulated bTB epidemic using the previously recorded RHT dates and random test dates (repeated every 4-years) revealed that the cyclic pattern observed under historical RHT was the results of seasonality and patterns of testing rather than the influence of weather conditions on the incidence rate (see Figure 3.4 and 3.5). A study by Wolfe *et al.* (2010) has also suggested that although seasonality was a risk factor for recurrent breakdowns, these seasonal effects were likely the results of seasonality in the testing and not due to the influence of weather patterns on bTB occurrence. Other studies suggests that weather conditions can affect the exposure of cattle to viable bacteria on pasture, hence increase the likelihood of environmental

infection, particularly in shaded conditions inside farm buildings and badger setts (Krebs, 1997; Phillips *et al.*, 2003). Though further comparisons of the simulation models under historical test dates and random test dates showed relative small differences in the total number of infected animals (and herds) at any given time point, and the speed of initial disease detection was also very similar.

Adjust timings of RHT

Four additional scenarios of the 4-year RHT were exercised in the simulation models, where tests would be clustered together (rather than at random) to focus on intensive testing of herds over a particular time period while maintaining the same level of surveillance effort (i.e. same number of RHT). Under all scenarios, the disease epidemic slowly spreads through the farm network with gradual increase in infection, before eventually settling into a stable stationary pattern. Over 500 simulations, the maximum level of infection (both in terms of infected animals and infected herds) within the network were not hugely different between different levels of test intensities (Table 3.2). The time of initial disease detection was generally faster when RHTs were conducted closer together compared to evenly distributed or at random, most importantly, the total number of new infections in the entire system was comparatively less as the intensity of testing increases. This was demonstrated by the larger reduction in infection number during the designated testing period (Figure 3.6), though this was also accompanied by a sharper increase in infected cases for periods outside of the testing window. It is worth noting, however, that adjusting the timings of the routine herd test on cattle farms appears to offer no real improvement in reducing the maximum epidemic level under current study, though the possibility of missing infection due to cattle movements should not be understated (i.e. infected cattle avoid being tested due to movement), particularly for High Risk Areas where pre- and post-movement testing are not implemented.

Different routine herd test intervals (frequencies)

The current bTB testing regime used throughout the UK involves regular one- or four-yearly testing of herds, though the majority of herds were tested on the four-year cycle (Defra, 2013a). The testing frequencies are associated with the epidemiological risk of the disease in the herd and in the surrounding geographical areas (Downs *et al.*, 2013). This non-random spatial and temporal variability in testing is an important aspect of the quality of surveillance for bTB (Green and Cornell, 2005). However, over the years, there were no published evaluation of the relative efficacy of different testing intervals. Under the stochastic bTB model, the disease is simulated with slaughterhouse surveillance only, and also in combination with routine herd testing at intervals of 1-year, 2-years, 4-years and 6-years respectively. Results from the minimal model indicate that slaughterhouse surveillance alone is not sufficient to eliminate or maintain the disease at a stable level as demonstrated also by Bessell *et al.* (2012a). While combined with an active surveillance strategy (i.e. regular routine herd tests), the disease can be controlled within certain limits. Implementing regular 6-year and 4-year whole herd testing resulted in substantial reduction (55% and 70%) in the maximum number of observed cases compared to the minimal surveillance scenario. And with increased surveillance effort (i.e. 2-year and annual herd testing) the disease can be maintained at a substantially lower level in the face of constant force of re-introduction. Though initial detection time showed small differences between the testing regimes, the epidemic pattern settles into a stable situation much quicker with more frequent testing. In any disease surveillance systems, a balance between effort and efficiency (resource management) must be considered. Moreover, an epidemic of bTB may be masked by the testing policy as illustrated theoretically by Medley (2003) who demonstrated that the reduction in test intensity may result in low numbers of detection, despite a potential increase in true (unobserved) prevalence and conversely, increasing the frequency of testing can lead to an

apparent epidemic because of increased detection (O'Reilly *et al.*, 2009). The RHT is the current screening test of bTB and data suggest that the majority of these tests are negative (Green and Cornell, 2005). Increasing the frequency of the RHT will increase the detection of infected cases and ultimately reduce the proportion of undetected infected herds (as shown by results from the present simulation models and also from Medley (2003)), though this is associated with a substantial increase in cost and effort.

Another significant cost, which is often neglected as is the case in the present model, is the imperfect specificity of the SICCT test. The specificity of the SICCT is currently estimated at approximately 99.8%. Generally speaking, the SICCT test is a fairly specific test, however, due to the large number of tests that is been carried out per year, it can still result in large number of false positives (i.e. 2 in every 10,000 tests). Under the current simulation model, specificity is assumed to be at 100%. This is because the objective of the model is mainly to simulate infection within- and between herds, as from epidemiological point of view, it is evident that false positive animals do not contribute towards disease transmission. Though the cost associated with the imperfect specificity should be accounted when evaluating the economic impact of bTB surveillance strategy.

Variation in within-herd transmission parameter (β)

In the present simulation study, the within-herd bTB transmission was simulated with an S-E-T-I state transition model with a daily time step, this structure has been adapted in many previous epidemiological studies (Barlow *et al.*, 1997; Fischer *et al.*, 2005; Conlan *et al.*, 2012). The rate at which susceptible animals become infected (S→E) from an infectious contact is governed by the within-herd transmission coefficient (β). The true underlying value of β depends on the specific population under study (Barlow *et al.*, 1997; Wahlström *et al.*, 1998; Munroe *et al.*, 2000) and is likely to vary across different farms due

to environmental conditions and factors associated with management practices (Barlow *et al.*, 1998; Perez *et al.*, 2002; Gopal *et al.*, 2006). My results from Chapter 2 also demonstrates this. As well as herd-level factors, it has been proposed that individual animals may vary in infectiousness and there may be the existence of super-spreaders that are inherently more infectious in the herd (O'Hare *et al.*, 2014) which can impact on the overall transmission rate. While it is difficult to accurately quantify the within-herd transmission rate for all infected herds, various levels of transmission were implemented in the simulation study to explore and evaluate the impact on the disease epidemic (in terms of infection size and time to initial detection) for a range of potential values of β . Compared with the baseline value (i.e. $\beta = 6^{-5}$ taken from estimates in Chapter 2), a ten-fold decrease in the transmission rate resulted in exactly ten-fold decrease in the maximum number of infected cases. However, a ten-fold increase in β value resulted in more than 15 times increase in the maximum number of infections. As β exceeds 6^{-4} (i.e. at this rate, 1 infectious animal can cause more than 20 new infections per year within a herd of 100 animals), the infection reaches more than 60% of the total population. Moreover, with the same level of surveillance effort, a faster transmission rate was more likely to result in earlier detection, although with larger number of cumulative infections. The upper and lower boundary of β values represents extreme case scenarios which are unlikely to be observed in field conditions given the slow spreading nature of bTB and the current national prevalence level (particularly in areas with high risk of bTB). Previous estimates are within the range from $6^{-6} - 6^{-4}$ with certain degrees of variability (Conlan *et al.*, 2012; O'Hare *et al.*, 2014; Brooks-Pollock *et al.*, 2014).

Variation in external force of infection α (background risks of disease introduction)

Another way susceptible animals can become infected is through external

factors that may include for example, inward cattle movements, contiguous spread from neighbouring herds or the presence of a wildlife reservoir (O'Hare *et al.*, 2014). These external routes of transmission were modelled through a single generalised infectious pressure, α . Similar to the value of β , estimation of α is also difficult and is dependent on many factors, hence, a range of values between 0 to 6×10^{-5} (new infections per susceptible animal per day) was applied in the simulation model. This rate is generally lower than the within-herd cattle-to-cattle transmission coefficient (β); however, the overall impact of α remains high, as α is active over the entire residence period of a susceptible animal in the herd, whereas an infectious animal is only active over its infectious period until removal due to death or export (Conlan *et al.*, 2012). The impact of α was demonstrated by results from the minimal model, where the external force of infection was completely eliminated (through 'isolation' with other herds), a large proportion of simulation runs did not develop into an epidemic (i.e. in more than 40% of simulation runs all infections were eventually detected within the pre-set time period). The maximum infected cases observed was only 4 animals (mean from 500 simulations) in the entire farm network (with $\beta = 6^{-5}$). Similar numbers were also observed for low levels of external infection. However, when α was $> 10^{-9}$, infection number started to build up and as it reaches $> 10^{-7}$ infection became wide spread with more than 60% of farms infected. Moreover, in more prevalent scenarios (due to high level of external infection), disease detection was rapid, as the infectious pressure from external sources become less significant, detection was more difficult with limited infection number. While the scenarios with zero or low level of external force of infection ($\alpha < 10^{-7}$) may be a representation for low disease areas in the UK (e.g. Scotland), where there is no evidence of established wildlife hosts, and risk from infectious import were generally small, in high risk areas the rate of disease re-introduction from external sources is likely to lie between 10^{-5} - 10^{-7} . This was broadly consistent with estimates from previous studies (O'Hare *et al.*, 2014; Brooks-Pollock *et al.*, 2014).

Although it is not possible to attribute the source of the external force of infection based on the model alone, infectious wildlife (i.e. badgers) are likely to be at least partially involved (Miller *et al.*, 2013; Byrne *et al.*, 2014). While both internal and external factors are important in driving the spread of bTB epidemic, in most herds, the force of infection owing to external causes is considerably lower than the within-herd force of infection suggesting cattle-to-cattle transmission is usually dominant (O’Hare *et al.*, 2014), however, the overall epidemic appears to be driven by the external force of infection (α maintains $R_0 > 1$). Previous low estimates for the role of inter-herd transmission in sustaining the national epidemic support the view that only a few herds are responsible for onward transmission to low risk areas, and that a self-sustaining cattle epidemic is unlikely (Green *et al.*, 2008). Thus, the balance of internal and external factors would suggest that any control programme must consider both maintenance hosts in order to succeed (O’Hare *et al.*, 2014). On the other hand, experience of bTB surveillance in many countries, including the USA, New Zealand and Canada, has shown that while *M. bovis* can be controlled when restricted to livestock species, it is almost impossible to eradicate once it has spread into ecosystems with free-ranging maintenance hosts (Nishi *et al.*, 2006; Fine *et al.*, 2011; Fitzgerald and Kaneene, 2013; Barron *et al.*, 2013). Therefore, it was suggested that focusing more efforts in prevention and control of *M. bovis* in wildlife may be the most effective way to mitigate economic and health costs of this bacterial pathogen (Miller and Sweeney, 2013).

Alternative diagnostic test sensitivities

When considering disease detection, the relative efficacy of slaughterhouse meat inspection (SMI) and routine herd testing using SICCT has previously been estimated using more extensive data on the time course of the epidemic (Conlan *et al.*, 2012; O’Hare *et al.*, 2014). However, there was considerable variability

in the individual test sensitivities due to factors such as the diligence of the tester in adhering to the correct testing procedure (Cousins, 2001), the within-herd prevalence of cattle sensitised to other non-TB environmental mycobacteria (Thom *et al.*, 2006), and factors that may alter the immune response to tuberculin of individual animals (Buddle *et al.*, 1994; de la Rua-Domenech *et al.*, 2006). Thus, depending on the population under study, a range of sensitivity estimates for SICCT may be applicable (Strain *et al.*, 2011). Therefore, the impact of different combinations of test sensitivity for SMI and SICCT test on the disease epidemic were explored in the simulation model. In addition, the effect of employing other forms of diagnostic tests (such as the gamma-interferon blood assay, or severe interpretation under the standard SICCT) for routine herd surveillance may also be incorporated by referring to their corresponding individual test sensitivities. Under the same testing regimes, models with higher test sensitivities improved disease detection and reduced the overall epidemic size. But the impact of improving SMI sensitivity was more substantial than improvements under SICCT test in the long term. This may be due to the fact that slaughterhouse surveillance was much more frequently applied over the period, and as infection reaches a certain threshold (close to maximum stationary point), SMI was more effective as a detection mechanism. Although the SMI was based on both the probability of inspection (i.e. proportion of cattle moving to slaughter every month) and detection (i.e. SMI sensitivity), it is likely to be dominated by the latter. This is because in the most common life history, cattle were directly moved to slaughter from the birth premises (Mitchell *et al.*, 2005; Vernon, 2011) and of those with more frequent movement histories, many were younger animals moving to low risk finishing units (Brooks-Pollock *et al.*, 2013). Though improving individual test sensitivity (for either or both SMI and SICCT) can lead to long term benefits in increased detection and lower the risk of epidemic potentials, significant savings can be made by reducing the extent of routine herd testing in low risk areas (while maintaining regular SMI) as the risk of onward transmission is low. In

addition, as indicated by previous findings, missed infections in those areas are likely to have little impact on the overall disease spread (Bessell *et al.*, 2012a; O'Hare *et al.*, 2014).

Impact of different contact networks on the disease epidemic

The study has suggested that improving detection of infection alone is not sufficient to eliminate recurrence of disease if the extrinsic infectious pressure acting on herds is not simultaneously addressed (Conlan *et al.*, 2012). One way in which healthy herds can become infected is through movement / trading of infected and undetected animals. Many previous studies have focused on the properties of the network that can be derived from these movements - treating farms as nodes and movements as directed (and potentially weighted) edges in the network (Vernon and Keeling, 2012). In the simulation models, disease spread was investigated and compared between random and scale-free contact networks with the same number of nodes, N , and average connections, E . For the same parameters of transmission and surveillance strategy, the final epidemic size on scale-free networks is substantially smaller than that on corresponding random networks. While in random networks, epidemic spread is much slower (i.e. taking longer to reach stationary point), scale-free networks has faster disease turnover time with rapid detections. This finding supports the suggestion by Kiss *et al.* (2006b) that cattle movement pattern with scale-free properties are more easily controlled and that when contact tracing is used to control an epidemic, scale-free network tracing can remove possible sources of infection with high average degree, hence limit the epidemic potentials due to the early and precise identification of most infected nodes (Kiss *et al.*, 2006a).

It was often been suggested that one important and intuitive feature of cattle movement networks (CTS) is that contacts between herds do not tends to occur at random. Factors such as farm production type (Ezanno *et al.*, 2006), farm

disease status (Weber *et al.*, 2006), trust between buyers and sellers (Von-Bailey and Hunnicutt, 2002), access to markets (Hobbs, 1997), and distance between farms (Lindstrom *et al.*, 2009) all contribute to the observed patterns of cattle movements. In particular, the significant costs associated with marketing and transportation over long distances tends to result in networks with *small-world* properties. This type of network property is characterised by the local clustering of contacts with the occasional long distant jumps that are responsible for bridging distant network communities (Watts and Strogatz, 1998). However, the current simulation model structure does not incorporate spatial element as part of herd-profile variable. The effect of small-world contact network may be the objective of future work by incorporating temporal and spatial information for each herd.

The present model also does not include other surveillance activities from current bTB control programmes in the UK, such as epidemiological contact tracing test, subsequent short-interval test following herd breakdown incident, pre and post-movement tests (Defra, 2005). These tests were also important control measures designed to prevent and reduce bTB spread and can be incorporated into the simulation models for future study. In addition, an age structure can be introduced in the model formulation to account for potential individual heterogeneities in disease susceptibility and transmission as well as modeling the changing herd dynamics which were neglected in the current model (Brooks-Pollock *et al.*, 2013; O'Hare *et al.*, 2014).

In a multi-host epidemic, such as bTB, it is vital that management and potential interventions are applied at the spatial and temporal scale on which the inter- and intra-species interactions occur (Pfeiffer, 2005). It is also important to consider the local ecological and epidemiological variables which interact in a particular system. Overall, the surveillance system for bTB must strike a balance between controlling three key processes: the rate of cattle-to-cattle transmission

within the herd, the infectious pressure acting to introduce infection into the herd from external sources and the removal of infection through different testing regimes. These are better addressed by integrated models that consider within- and between-herd transmission as well as the ability to implement and rehearse different control strategies. Simple models such as one presented here are of course a caricature of the true epidemiological situation; individual herds will vary in structure and composition combined with complex and changing control policies, therefore may not accurately predict the actual outcome of future incidence.

However, the current simulation framework provides a very useful basis to assess the relative contribution of different transmission mechanisms to bTB spread and the relative efficacy of the corresponding control measures under field settings. These features can provide policymakers with valuable information on how to allocate limited disease control resources more effectively over the course of an eradication programme. This is particularly important since evaluation of alternative surveillance strategies is very difficult to compare realistically in any other way. Moreover, by analysing disease spread with a range of different epidemiological parameters, these models can potentially help policy makers to solve the inverse problem of what the epidemic would look like given the underlying disease parameters and control strategies. Although simulation models such as this are challenging to parameterise due to the lack of empirical data on the within-herd structure and transmission dynamics, they represent an important direction for future epidemiological research. By tailoring the control strategies to the underlying bTB spread, it is possible to provide farmers, veterinarians, and policy makers with better guidance on the optimal strategies for disease control and prevention. These models can also be linked with traditional network simulation models to explore issues such as the effects of animal and herd demographic characteristics on the sensitivity of national disease surveillance programmes.

Chapter 4

Potential impact of recent calving on response to the standard SICCT test for bovine tuberculosis in cattle

4.1 Introduction

The diagnosis of bovine tuberculosis (bTB), like human tuberculosis, remains an extremely challenging task worldwide. There is currently no single test which can reliably identify all infected animals (Bourne, 2007; Karolemeas *et al.*, 2012). Traditionally bTB infection in cattle is diagnosed in live animals on the basis of delayed hypersensitivity reactions to intradermally injected mycobacterial antigens (OIE, 2009). This test is the standard method used for bTB detection worldwide and is the prescribed test necessary for clearance to trade

internationally. In Great Britain (GB) and Northern Ireland, the single intradermal comparative cervical tuberculin (SICCT) test (also known as skin test) is the primary diagnostic tool for the identification of tuberculous cattle as part of national surveillance programmes (de la Rua-Domenech *et al.*, 2006). The test compares the immune response of individual cattle to bovine (*Mycobacterium bovis*) and avian (*M. avium*) mycobacterial antigens, and depending on the relative degree of reaction after 72 hours, animals may be classified as positive (R), inconclusive (IR), or non-reactors (N) (Green and Cornell, 2005). In October 2006, the gamma-interferon blood assay, a highly sensitive laboratory based test was introduced and is currently being used in conjunction with the SICCT test under specific circumstances to improve the chances of detecting infected cattle at early stages of infection. The aim is to rapidly reduce bTB incidence in high prevalence areas to minimise the risk of bTB becoming endemic in the local wildlife (Defra, 2008a).

From previous investigations, it was shown that the reactivity to the SICCT and gamma-interferon test is dependent on a range of variables, such as the nutritional status, masking infection (e.g. *M. avium-intracellulare* including *M. avium paratuberculosis*), concurrent infections (e.g. parasitism) and time-from-infection (Monaghan *et al.*, 1994; de la Rua-Domenech *et al.*, 2006). More specifically, there is substantial evidence that the sensitivity for detecting infected animals using SICCT appears to be lower for cattle in the early stages of infection or for cattle experiencing concurrent illness, or poor management conditions (Costello *et al.*, 1997; Clegg *et al.*, 2011b). Analysis by Brooks-Pollock *et al.* (2013) demonstrated that bTB incidence rate has a strong age dependency and that the probability of detecting infection increases with age. Moreover, animal age was considered a potential confounding factor for being identified as a reactor on routine herd test (RHT) in many previous studies (Gates *et al.*, 2013; Brooks-Pollock *et al.*, 2013; Munroe *et al.*, 2000), although there is an important

distinction between test results and true infection rates. Infectious diseases in general arise from an interaction between the infectious agent, the host, and a range of covariables. Risk factors such as biological, behavioural, environmental or genetic differences are known to influence both transmission and susceptibility (Reilly and Courtenay, 2007; Skuce *et al.*, 2012). It was suggested that there was a strong association between animal breed and SICCT test outcome, with smaller reactions (difference in skin test measurement after injecting bovine and avian tuberculin) in common dairy breeds such as Jersey, Friesian and Holstein, and larger reactions in various beef breeds and their crossbreds (Amos *et al.*, 2013).

As well as the confounding factors mentioned above, it has also been suggested that the ability of the tests to identify tuberculosis-infected animals could be affected by concurrent stress, particularly when cattle are tested around the time of parturition. A study by Kerr *et al.* (1946) observed that many infected cattle failed to react on SICCT tests administered post-partum, but subsequently became positive reactors when the test was repeated 4 to 6 weeks later (Strain *et al.*, 2011). Although the results from this analysis must be interpreted with caution given evidence that repetitive SICCT testing within short intervals may induce desensitisation, making the effect of post-calving less distinguishable (Radunz and Lepper, 1985; Coad *et al.*, 2010). The gamma-interferon assay is not known to suffer from this disadvantage, and experiments by Buddle *et al.* (1994) found that cattle tested using the gamma-interferon test also experienced a temporary reduction in immune response within two weeks of calving. The innate and acquired immune defence mechanisms appear to be weakest from week 3 prepartum to week 3 post-partum when dairy cattle experience immunological and physiological stress due to parturition, changes in their environment and poor energy balance (Mallard *et al.*, 1997, 1998), and stress related endocrine changes (Kimura *et al.*, 1999). This may depress the immune response to mycobacterial antigens leading to decreased test sensitivity.

While there have been many studies investigating confounding factors such as co-infection (Flynn *et al.*, 2009), time from infection and desensitisation due to repetitive testing (Thom *et al.*, 2004; Conlan *et al.*, 2012), little is known about the effect of stress-related factors such as pregnancy and calving on the sensitivity of the SICCT test and the gamma-interferon assay. In this chapter, a matched case-control study was performed to determine whether calving events are associated with lower detection rates (test positiveness) under gamma-interferon and SICCT test on confirmed breakdown herds (where at least one test positive animal were identified with TB-like lesion at slaughter or was culture positive) in high-incidence parish testing areas (i.e. annual and two yearly testing parishes). The analysis accounts for confounding factors by matching individual cases (animals that reacted positively to SICCT on initial routine herd test) with controls (non-reactors on initial routine herd tests) based on farm of residence to account for differences in management practice, animal age to account for heterogeneities in disease exposure and breed to account for genetic variations in heritability and susceptibility to disease. Conditional logistic regression models were applied to analyse the matched dataset to establish the relationship and potential effect between recent calving on test positiveness.

4.2 Materials and methods

4.2.1 Datasets

The results of all *ante-mortem* bTB testing and suspected or confirmed cases identified through slaughter surveillance were contained in the Sam's IT system, which is collated and managed by the Animal Health and Veterinary Laboratories Agency (AHVLA). At a herd-level, surveillance histories with

summary information on the number of cattle tested, total number of animals in the herd, date, type of test and administrative information such as farm (CPH - county parish holding) and test (“test id”) identification number were also recorded. In records where a positive reactor, inconclusive reactor, or slaughterhouse (SLH) cases (i.e. animal with visible lesions) was identified, detailed information for the animal including passport number, residency farm location and results from *post-mortem* diagnostics was entered in the “Animal” table in the Sam’s IT system along with any follow-up test or actions taken. In addition, the event of detecting one or more test positive animals, or *M. bovis* cultured from lesions detected at the slaughterhouse, triggers a herd breakdown. Consequently a unique breakdown of records is created in the “breakdown” table in the Sam’s IT system with a breakdown incidence number attached. This is subsequently linked with the herd testing history and reactor information (in “animal” table). A breakdown herd is classified as “confirmed” breakdown when at least one or more reactors is detected with visible TB-like lesion at slaughter or was culture positive at *post-mortem* laboratory test. Demographic and movement information for individual cattle present on the study farm at time of testing, as well as the cattle that tested positive were traced using Cattle Tracing System (CTS) database, run by the British Cattle Movement Service (BCMS). The age, sex, breed classification, and calving histories of each animal were recorded. The Sam’s IT system and the CTS database each have different standard for recording information on individual cattle and herds. “CPH” number in the Sam’s IT system was matched to the “location id” in the CTS database to identify herds information, and animal “ear-tag” (passport) number from the Sam’s IT system was linked to “livestock id” in the CTS to identify details of individual cattle.

For the current analysis, data from the Sam’s IT system covering bTB tests between 01 January 2006 to 31 December 2011 were extracted, and CTS data from January 2002 to December 2011 were used (data on CTS pre-2002 were considered

incomplete). All datasets were extracted using PostgreSQL (Anonymous, 1996) with data analysis and statistical modelling carried out in R (R, 2012).

4.2.2 High incidence testing areas

Locations within England and Wales were classified into bTB-risk groups based on the frequency of routine herd test for cattle farms within the parish (Gates *et al.*, 2013). A high incidence region was taken to be one where the parish testing interval (PTI) was 12 or 24 months and was subject to annual or two yearly whole herd test of all cattle over 6 weeks that are present on the date of the test. A low incidence region has a PTI of 36 or 48 month and is subject to a less frequent routine herd test with exemptions on certain cattle, which were not required to be tested. Given that parishes rarely change from high incidence to low incidence testing interval, the list of PTIs recorded in CTS for 1st quarter 2008 was taken as snapshot criteria to determine the location risk in the study.

While positive results were recorded at individual animal level, negative test results were aggregated at herd level in the Sam's IT system, in order to determine negative tests at the animal-level, cattle present on herd prior to testing need to be assessed against Defra's eligibility criteria. Based on bTB testing guidelines, cattle over six weeks of age on farms within high incidence areas were all eligible to be tested within the routine whole herd test (WHT - 12 or 24 months). In low incidence regions however, several criteria apply, only cattle that are recently purchased, intended for breeding or previously calved were eligible under the routine surveillance test. For these reasons, all farms within the high incidence testing parishes (PTI = 12 or 24 months) were selected and their breakdown records between 01 January 2006 to 31 December 2011 were extracted from the Sam's IT system. Moreover, for the purpose of this analysis, only confirmed

breakdown herds (at least one reactor with visible lesion observed at *post-mortem* or positive *M. bovis* culture) identified via routine WHT (annually or two yearly) were selected as the study population. Reactors identified through other test types such as pre and post-movement testing, follow up testing, tracing, etc, were excluded to control for previous exposures to bTB antigen and other factors that may potentially interfere with the test outcome.

4.2.3 Animal selection and case definition

Positive test results in each breakdown were collated directly from the “Animal” table in the Sam’s IT system, which enables tracing of movement and demographic information as well as to calculate relevant risk factors. Reactor cattle were identified by the test results “R” (under both SICCT and gamma-interferon), while inconclusive reactors were denoted as “IR” (specifically for SICCT). For the purpose of this analysis, animals slaughtered due to direct contact with infected animals or slaughterhouse cases (SLH) were ignored, and IRs were analysed as a separate analysis. From each confirmed breakdown episode (i.e. between initial breakdown date until eventual removal of breakdown status), Rs and IRs that fulfilled the following criteria were identified as the case group.

1. Female cattle born in year 2002 - 2011.
2. Detected during the disclosing WHT (initial test that identified bTB reactor or suspected cases that initiated the breakdown investigation) under standard interpretation (reactors from subsequent follow up tests were not considered).
3. At least 24 months old at the time of test (few animals calve prior to 24 months of age).

4. Home-bred animals with no previous movement history (eliminate potential exposure due to movements between other farms).

All confirmed breakdown herds, which contained at least one reactor (a case) from the above selection criteria, were included in the analysis. It was fairly easy to deduce the animal's individual characteristic such as sex, breed and age in days (test date minus birth date) by linking the ear tag number with demographic information in the CTS database. However, the distinction between home-bred or purchased animal and the disclosure of reactors during the initial routine WHT (rather than detections made in subsequent testing), needed to be inferred through data manipulation and cross referencing between the CTS database and the Sam's IT system as shown in Figure 4.1. The following sequential steps assist the schematic flow chart in Figure 4.1 and briefly describes the process to determine reactors identified in selected herds.

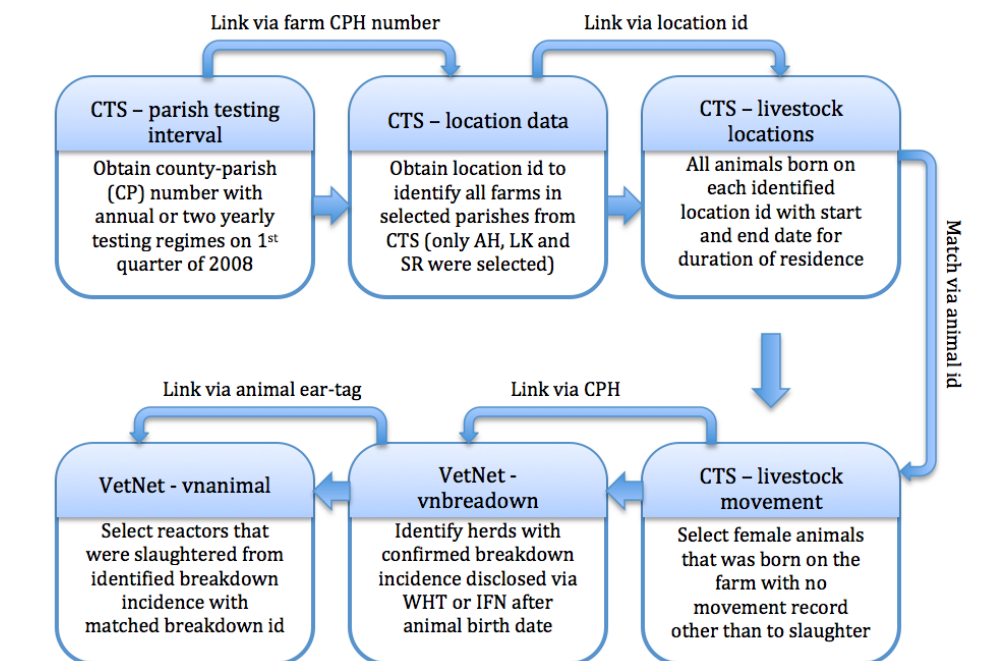


Figure 4.1: Schematic flow chart showing the process of data extraction and manipulation

1. All cattle farms in high incidence regions (annual or two yearly parish testing areas) according to the pre-assessed routine test frequency requirement by Defra during the 1st quarter of 2008 were selected (only locations with AH - agricultural holding, LK - landless keeper and SR - slaughterhouse red meat were used in the analysis).
2. Home-bred female animals born between 2002 - 2012 on identified farms in high incidence areas were selected. Only animals with movement records to slaughterhouse (direct or via livestock market) were retained (i.e. home-bred animals that were never moved away).
3. From the list of farms identified in high-risk areas, herds which have breakdown incidences recorded in the Sam's IT system between 2006 and 2011 and confirmed through *post-mortem* examination or positive bacterial culture were selected. Moreover, the initial disclosing test was restricted to either "VE-WHT", "VE-WHT2" or "VE-IFN" from the Sam's IT system breakdown table. This was used to indicate herd breakdowns disclosed via annual routine WHT, two yearly routine WHT or gamma-interferon test respectively.
4. Home-bred animals present on herd during the breakdown episode were selected and records of their individual characteristics such as breed, sex and age.
5. Test positive animals (reactors) during the breakdown episode were distinguished from test negatives using "Animal" table in the Sam's IT system where information on all reactors were recorded.
6. The Sam's IT system was then used to further restrict reactors by removing those that were detected at a later date in relation to the initial breakdown date, due to the more stringent interpretation of the skin reaction used in follow-up testing for a herd breakdown (Krebs, 1997) (i.e. "test id"

is uniquely assigned to individual test scenario within each herd and reactors identified under different “test id” to the initial disclosing test were subsequently removed).

7. Finally, animal calving history were obtained from the CTS “livestock relationship” table.

4.2.4 Control selection

The control group consisted of all test negative cattle within the confirmed breakdown farms that also met the above four selection criteria in the case definition. In general, there is no historic information in the Sam’s IT system about cattle that tested negative to the SICCT test (Brooks-Pollock *et al.*, 2013). All test negative cattle that were present on the farm at the time of testing were inferred by combining animal-level data in CTS records with herd-level testing data in the Sam’s IT system. Further assumptions were made that all cattle present during a test that fell within the selection criteria were tested, since according to Defra’s eligibility guidelines, all animals over 6 weeks old are eligible for WHT. Specifically, all confirmed breakdown herds with at least one case (reactor) identified through the selection criteria were reconstructed. The breakdown date (given as the animal test date) along with farm location (CPH) number and the unique breakdown incidence id were extracted from the Sam’s IT system breakdown table (Figure 4.1). Then the CPH number was matched to the CTS location table to obtain a CTS location id. Using this CTS location id, a list of cattle id numbers that were born on the premises (during or after year 2002) before the breakdown date and never moved away (prior to the breakdown test) from the CTS “movement table” was extracted. The passport number, birth, death date and breed classification of each animal were also extracted from the CTS database using the animal id number. Using the same procedures as

the case selection, animals that did not meet the pre-determined criteria were removed from the resulting dataset.

All eligible animals present on farm, other than the ones in the case group, were assumed to be test negative on the initial testing date (and therefore included as controls), this includes all subsequent reactors and inconclusive reactors detected during follow-up testing procedures (i.e. control animals that were tested negative during the initial WHT but were subsequent reactors).

4.2.5 Matching criteria

Once the control samples had been selected, all cases in the same breakdown incident were matched with a list of controls in a one-to-many matching relationship. Each case was matched to as many controls as possible based on the unique breakdown incidence id (same herd may have multiple numbers of breakdown incidences), breed and age (± 90 days of the age of the case animal). For example, a 36-month-old homebred female Holstein Friesian detected as a reactor during a routine WHT was matched with a list of homebred female Holstein Friesians, that tested negative on the same farm during the same test, and was born 90 days prior to or after the birth date of the reactor. Each case was matched with as many controls as possible to increase the sample size and to reduce heterogeneity and bias that would result from ignoring unmatched (but eligible) animals. Moreover, matching was carried out on a one-by-one basis to reduce the clustering effect of grouped (or batched) matching. Specifically, one random control was matched with one case animal at a time based on the matching criteria, the remaining list of controls was then matched randomly to the remaining cases retrospectively until there are no more controls that could be matched. The set of matched case

and controls were given a unique match id to indicate that they belong to the same matched group. The matching was carried in R software (R, 2012).

The process of identifying/matching case and control samples were then repeated for breakdown farms detected through gamma-interferon blood test. The gamma-interferon test was also classified as whole herd test according to Defra's eligibility guidelines (all cattle aged 6 weeks or older are subject to test), however, from this point forward, WHT refers specifically to whole herd SICCT test under standard interpretation.

4.2.6 Explanatory variables

The explanatory variables calculated on each set of cases and controls in this study are listed in Table 4.1. The binary variable "recent calve" was defined as 1: cattle giving birth to calve(s) within 60 days prior to the positive WHT, and 0: cattle calved more than 60 days prior to the failed WHT or that had no previously recorded calving. Continuous explanatory variables (days since the most recent calving, and the number of previous calving events in relation to the failed WHT date) were each converted to categorical groups described in Table 4.1. In addition, an extra factor variable "week" was introduced to further examine the effect during the period immediately after recent calving (60 days was equally divided into two weekly bins).

Female animals that had calved were identified using the relationships data in the CTS extracts, which records the dam id and calf id along with the corresponding birth date. Explanatory variables related to calving events for each animal were calculated based on the most recent calving instance. For example, "days since previous calving" were calculated as the difference in days between

Table 4.1: Explanatory variables calculated for each animals in the case-control analysis

Explanatory factors	Code / Values	Description
Recent calving	0	Calving more than 60 days prior to the breakdown or never calved
	1	Recent calving within 60 days prior to the breakdown
Days (since most recent calving)	0	Animals never calved
	1	Animals calved more than 100 days
	2	Animals calved within 100 days
Previous calvings (Parity)	0	Animals never calved
	1	Animals with 1 previous calving at the time of breakdown
	2	Animals with 2 previous calvings
	3	Animals with 3 previous calvings
	4	Animals with 4 previous calvings
	5	Animals with 5 previous calvings
	6+	Animals with 6 or more previous calvings
Weeks	0	Animals calved more than 8 weeks or never calved
	2	Calved within 6-8 weeks
	4	Calved within 4-6 weeks
	6	Calved within 2-4 weeks
	8	Calved within 0-2 weeks

animal test date (the initial failed WHT) and the most recent calf birth date prior to the test. The number of previous calves (commonly known as parity) was aggregated up to the point of the failed WHT test date.

4.2.7 Statistical analysis

Due to the nature of a matched case-control study, conditional logistic regression models (Craiu *et al.*, 2008) were developed to explore the associations between the various factors of interest and testing positive under the specific diagnostic test (namely, SICCT and gamma-interferon). The two tests were analysed separately for 1) animals with recent calving event and 2) animals with no calving history or non-recently calved between 2006 to 2011. Several variables were used to define the proximity between the most recent calving and the reference diagnostic test. Recent calving within 60 and 100 days were analysed independently as binary explanatory variable. While categorical variable “week” was created to test for potential trend over 8 weeks period post-calving. Many covariates described in Table 4.1 are proxies for each other, so due to underlying dependent correlations (e.g. colinearity between week, days and recent calve), univariable conditional logistic regression analyses was performed on each covariate in turn with test results (0: negative or 1: positive) as the binary outcome variable.

Under the conditional maximum likelihood estimates, the resulting odds ratio along with the 95% confidence interval of the independent variables associated with the outcome were reported. Associations with a p-value < 0.05 were considered statistically significant. Match id was used in the model formulation to segregate the data into strata so that the association of within-strata exposure (calving) and outcome (test results) can be incorporated.

4.2.7.1 Sensitivity analysis

A series of sensitivity analyses were performed to analyse the effect of calving related activity on the test outcome based on different case and control definitions. During the case and control selection procedure, information on inconclusive reactors in each breakdown herd were also extracted along with details for each reactor. In the first sensitivity analysis, IRs were combined with the reactor animals (they were effectively treated as test positive) as cases, and were compared with test negative animals, and then IRs was separately compared with test negatives and reactors respectively in the conditional logistic regression model, to test the effect of relevant covariates under different case and control definitions.

A second sensitivity analysis were conducted to investigated the effect of selecting animals with different calving histories on the outcome of the test and whether the effect of recent calving was consistent across different sub-populations. The data were restricted to animals with a history of at least one calving and this was followed by analysis on animals with exactly one calving event to examine the variation in the effect using conditional logistic regression with the same set of explanatory variables.

4.2.8 Gamma-interferon analysis

The gamma-interferon assay based on a blood sample was only widely applied in the UK since October 2006. Current bTB testing regulation states that this type of test is mandatorily applied on SICCT test negative animals in all confirmed new bTB incidents within low risk areas. However, in areas of high bTB incidence, gamma-interferon was only applied on a discretionary basis, normally when persistent confirmed infection fail to resolve through repeated SICCT tests,

or on SICCT test negative animals in severe bTB incidents, to inform decisions around whole or partial herd slaughter (Defra website). Therefore, the analysis was restricted to periods that had the most available testing data (i.e. from 2006 to 2009).

“VE-IFN” was used in the Sam’s IT system’ breakdown table to indicate herds disclosed for infection via the gamma-interferon test, and this was used to select the target population of farms. Breakdown herds disclosed via “VE-IFN” normally had one or several inconclusive SICCT test results previously, indicating perhaps infection was in early stage and therefore less likely to test positive to the standard SICCT test. Hence, the effect of calving related activity on confirmed breakdown herds identified through gamma-interferon blood test was analysed separately, but follow the same methodology as the routine SICCT test described above.

4.3 Results

4.3.1 Case and control samples

In the 1st quarter of 2008, 3,727 (31%) parishes were high incidence testing areas, predominately in southwest England and the majority of Wales (see Figure 4.2). This included a total of 103,828 individual farm holdings with more than 12 million animals born since 2002. Almost 3 million female animals have been classified as home-bred on nearly 30,000 unique farm holdings using the selection criteria. This sample was significantly reduced after matching and selecting for breakdown herds detected through routine WHT between 2006 and 2011.

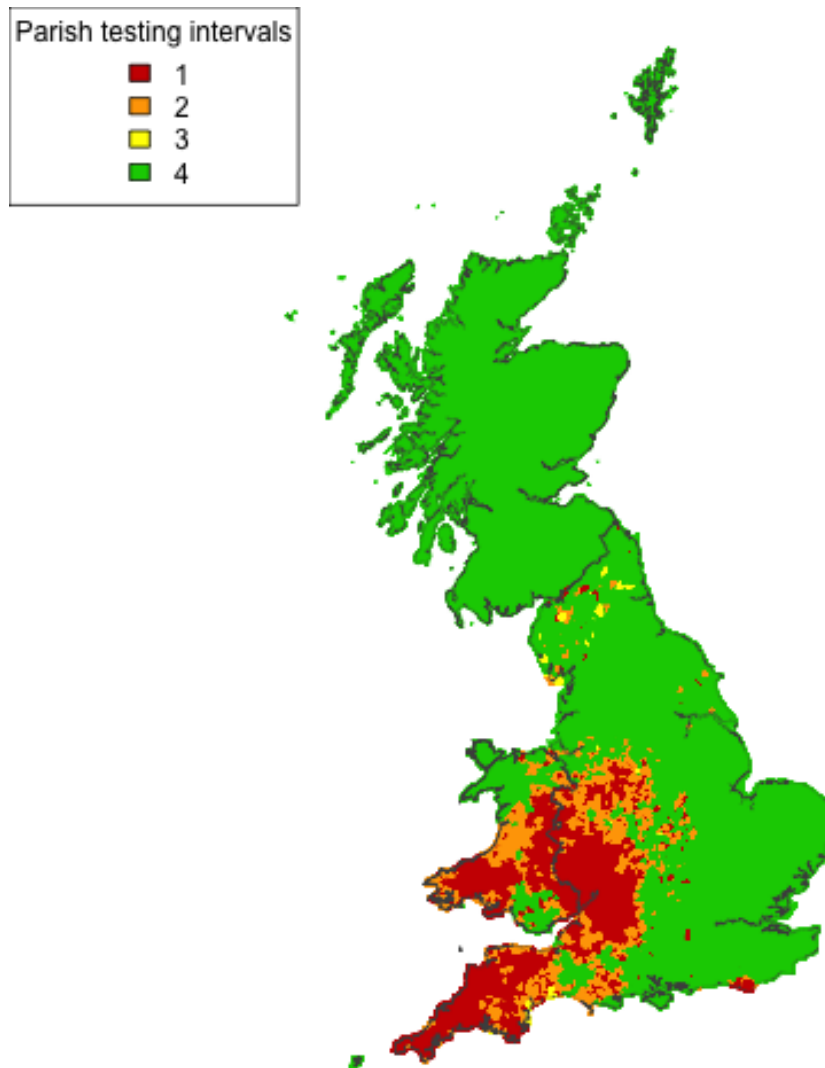


Figure 4.2: Study regions and information with different Parish Testing Intervals (PTI) in the UK in 2008. Red colour indicate annual testing parishes, orange for 2-yearly testing parishes, yellow for 3-yearly testing parishes and green regions are 4-yearly testing areas

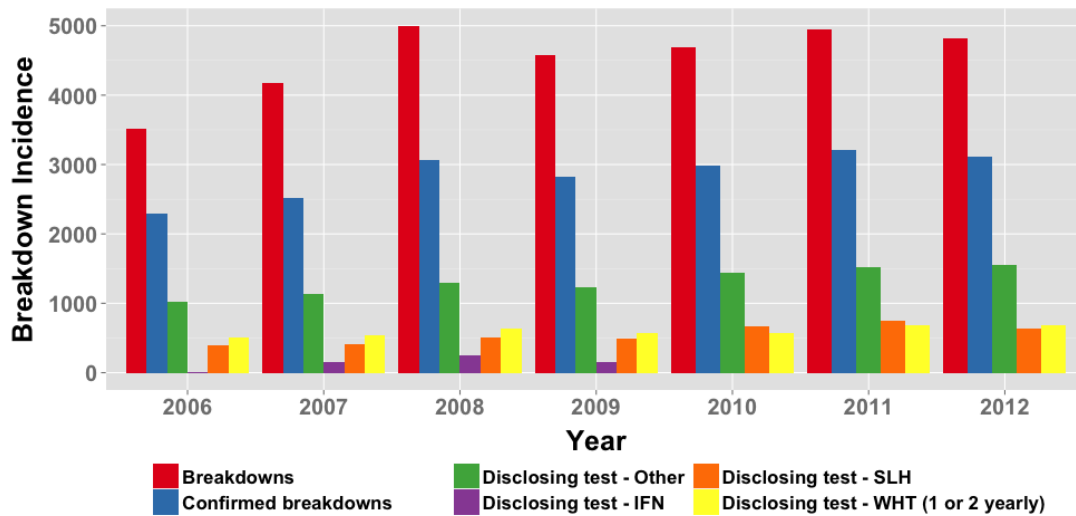


Figure 4.3: Number of breakdown and confirmed breakdown incidences between 2006-2012 in the study region (high incidence testing parishes), aggregated by the initial disclosing test type (i.e. how the breakdown were identified)

The majority (63%) of breakdowns were confirmed at *post-mortem* by detection of visible lesions or by culture of *M. bovis*. In addition, more than a quarter of all confirmed breakdowns were detected via WHT during each year (Figure 4.3), this resulted in 4,284 unique breakdown incidents being selected in total that contained animals which meets the case and control definition. These confirmed breakdown herds included 2,914 cases (reactors) and 158,286 controls (test negatives), along with 2,382 inconclusive reactors (analysed separately, see section on sensitivity analysis), detected during the initial breakdown test. The average percentage of recently calved (within 60 days of breakdown test) animals within the case and control populations were 7% and 8% respectively, across the years. In addition, more than 70% of cattle selected had at least 1 previous calving prior to the breakdown test.

The resulting case and control sample in year 2006 - 2011 based on matching criteria of breakdown incidence (accounts for farm location as well as each unique breakdown occasion), breed and age was shown in Table 4.2.

Table 4.2: Matched case and control samples with calculated proportions of recently calved animals (60 and 30 days respectively) for year 2006 - 2011

Year	bTB case animals			Control samples		
	N	% Recently calved (60 days)	% Recently calved (30 days)	N	% Recently calved (60 days)	% Recently calved (30 days)
2006	176	8%	2.9%	1208	9.9%	4.6%
2007	306	9.4%	5.8%	1898	12.1%	5.9%
2008	393	9.4%	2.1%	1957	10%	4.6%
2009	354	6.5%	3.4%	2040	10.7%	5.5%
2010	262	9.5%	4.6%	1770	13.6%	6.7%
2011	382	6.8%	3.1%	3026	10.3%	6.1%

Based on the matching results in Table 4.2, the mean match success rate was 11.4% from pre-matched data (i.e. a successful match is where at least one control is matched with one case) across the 7 calendar years. The proportion of animals exhibiting the risk factor (i.e. recent calving) was slightly different between case and control samples. The mean proportion of case animals that recently calved (within 60 days and 30 days) prior to being tested was 7.2% and 2.8% respectively, while recently calved animals in control group was slightly higher at 9.8% and 4.6% respectively.

4.3.2 Calculating risk factors

The explanatory variables described in Table 4.1 were calculated for each animal based on their individual characteristics at the time of the initial breakdown test. Overall, 15,205 controls were matched with 2,284 cases. 2011 identified the highest number of control samples, while case group have steadily increased

over the time period (Figure 4.4 a). Recently calved animals (within 60 days) constituted around 9% of the total sample (Figure 4.4 b). Moreover, when the 60 days period was equally divided into two-weekly categories, the proportion of recently calved animals was approximately equally distributed between each category. Among the selected samples, more than 50% of the animals had 1 or 2 previous calving events and only 8% had more than 3 calvings history (Figure 4.4 c). For breeding cattle that had at least one previous calving event, the majority calved within one year of the breakdown test, with few exceptions of old breeders that gave birth more than 2 years prior to the breakdown (Figure 4.4 d).

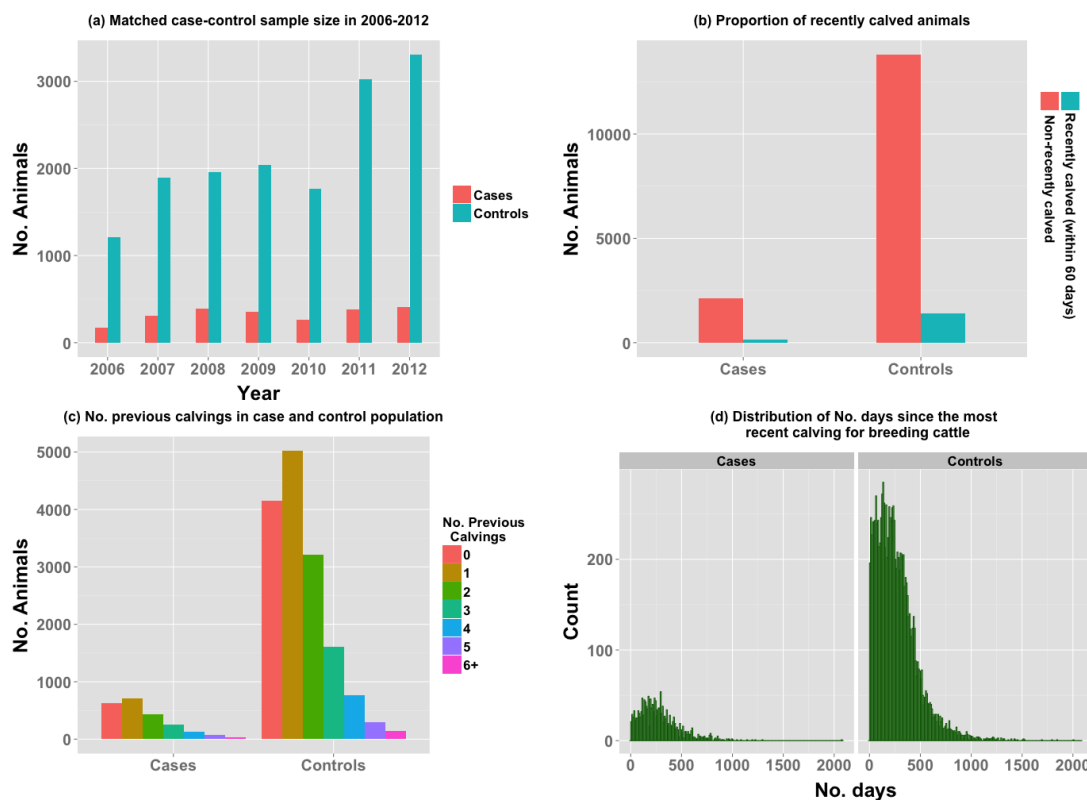


Figure 4.4: Summary plots of variables of interest for matched case and control a) sample size across the 7 calendar years, b) proportion of recent calving, c) number of previous calving events and d) distribution of the number of days since the most recent calving

4.3.3 Conditional logistic regression analysis

Each explanatory variable was analysed in turn under the univariable conditional logistic regression model framework with “match id” representing each unique set of matched samples. Recent calving prior to the reference test (compared to non-recent calving or not calving) was significantly associated with a decreased odds of reacting to the SICCT test. Specifically, SICCT conducted on animals during 60 days post-partum period were 0.72 (95%CI: 0.59 – 0.87, p -value < 0.001) times less likely to test positive in comparison with animals with no previous calving history or calved more than 60 days prior to the test. While using animals with no previous calving history as the baseline category (i.e. previous calving = 0), the odds of testing positive during a test is 0.85 times less likely for animals calved within 100 days. Conversely, calving more than 100 days were 1.22 times more likely compared to animals which never calved. Although a noticeable change of signs in the odds ratio estimate between categories in variable “days”, only the latter category were statistically significant (Table 4.3).

Furthermore, there was a decreasing trend over the post-parturient period in the odds of animals testing positive under the standard diagnostic test, with the strongest non-reactivity effect (smallest odds ratio) for tests that were conducted closer to the calving date. From Table 4.3 the “week” variable showed that testing within 2 weeks post-calving were 0.66 (95%CI: 0.45 – 0.99, p -value < 0.043) times as likely to test positive. There is an increasing trend of odds ratio estimates in subsequent categories (the further away from calve date) with calving between 6-8 weeks showing the least protective effect with estimate of 0.88 (95%CI: 0.47 – 0.98, p -value < 0.036). There was also a general reduction in the risk of SICCT test positive for each increase in the number of previous calving (parity), however, these categories was all statistically insignificant.

Table 4.3: Results of univariable conditional logistic regression analysis showing the effects of calving related variables on the odds of a positive SICCT test results

Explanatory factors	Odds ratio (95% CI)	p-value	N cases: controls
Recent calving (within 60 days)			
No	1		2128 : 13810
Yes	0.72 (0.59, 0.87)	< 0.001	156 : 1395
Days (since most recent calving)			
0 (Never calved)	1		631 : 4157
1 (<= 100 days since calving)	0.85 (0.69, 1.05)	0.105	273 : 2379
2 (>100 days since calving)	1.22 (1.02, 1.44)	0.026	1380 : 8669
Previous calves			
0	1		631:4157
1	1.14 (0.96, 1.35)	0.124	709 : 5018
2	0.95 (0.77, 1.18)	0.655	436 : 3212
3	0.95 (0.73, 1.25)	0.738	261 : 1612
4	0.85 (0.6, 1.2)	0.344	132 : 765
5	1.01 (0.64, 1.6)	0.961	78 : 297
6+	0.72 (0.38, 1.35)	0.305	37 : 144
Week			
0 (Non-recent or never calved)	1		2128 : 13810
2 (calved between 6-8 weeks)	0.88 (0.47, 0.98)	0.036	39 : 413
4 (calved between 4-6 weeks)	0.79 (0.54, 1.13)	0.195	40 : 335
6 (calved between 2-4 weeks)	0.74 (0.52, 1.07)	0.109	42 : 321
8 (calved between 0-2 weeks)	0.66 (0.45, 0.99)	0.043	35 : 326
Total			2284 : 15205

4.3.4 Gamma-interferon analysis

Gamma interferon blood test was first applied in the UK during October 2006, this was reflected by the low number of breakdown incidence identified via “VE-IFN” (Figure 4.3) prior to 2007. Due to the discretionary nature for requirement of the test, 2007–2009 saw a substantial increase in breakdown incidence detected by gamma-interferon (normally after previous inconclusive SICCT test results), but the trend for using this type of test as a supplement declined by year 2010–2012. Overall, 1,578 unique breakdown incidences were detected via gamma-interferon blood test between 2006–2009 in high risk areas. Approximately 40% (677 breakdowns) were found with a matched case and control using the selection criteria. The final matched dataset contained 924 cases and 7,619 controls, while the percentage of recent calving were slightly higher in comparison to the SICCT test analysis at 10.2% and 8.5% from case and control samples, respectively.

Results from the univariable conditional logistic regression analysis are shown in Table 4.4, variable “days” and “previous calving” were statistically non-significant and therefore not reported. From the model outcome, similar conclusions can be drawn for the effect of recent calving on gamma-interferon blood test. Animals which had a recent calving within 60 days were 0.77 (95%CI: 0.59 – 0.99, p-value < 0.048) times less likely to test positive compared to animals without calving recently or no previous calving. A trend of decreasing odds ratios were also observed in the “week” variable with increasing time since calving. However, compared with the standard SICCT test, the effect seems to have stronger influence on the test outcome for periods immediately after calving, and for a short lived period beyond 4 weeks. Categories for calving between 0-2 and 2-4 weeks were both statistically significant, suggesting that for tests conducted within 4 weeks post-calving, animals were approximately half as likely to react

to the gamma-interferon test in comparison with animals who have not recently calved, or never calved during their lifetime. But this effect seems to diminish beyond 4 weeks, evidently shown by the non-significant odds ratio estimate and a change of effect in the odds ratio for animals calved more than 6 weeks prior to the test.

Table 4.4: Results of univariable conditional logistic regression analysis showing the effects of calving related variables on the odds of a positive gamma-interferon test result

Explanatory factors	Odds ratio (95% CI)	p-value	N cases: controls
Recent calving (within 60 days)			
No	1		845 : 6843
Yes	0.77 (0.59, 0.99)	0.048	76 : 776
Week			
0 (Non-recent or never calved)	1		845 : 6843
2 (calved between 6-8 weeks)	1.22 (0.8, 1.87)	0.355	30 : 183
4 (calved between 4-6 weeks)	0.87 (0.53, 1.41)	0.565	22 : 189
6 (calved between 2-4 weeks)	0.5 (0.28, 0.9)	0.021	14 : 209
8 (calved between 0-2 weeks)	0.47 (0.24, 0.91)	0.025	10 : 195
Total			921 : 7619

4.3.5 Sensitivity analysis

Table 4.5 shows the effect of calving related activity on different case and control definitions (i.e. with inclusion of inconclusive reactors or cattle with at least one previous calving), and also whether the non-reactivity effect of calving varies with different threshold for closeness to the calving event (i.e. 30 days rather than 60 days).

Firstly, when inconclusive reactors were treated as test positives and combined with the reactor animals (as the “case” group), the effect of recent calving, on the combined data, exhibits quantitatively similar results as for the reactor only analysis (Table 4.3), but the effect extends up to 4 weeks (compared to just 2 weeks) since previous parturition (the latter categories of “week” were both statistically significant). The variable “days” has shown a change of sign and was only marginally significant on the second category. However, when inconclusive reactors were separately compared with SICCT test negatives and positive reactors respectively, the resulting conditional logistic regression model failed to identify any significant risk factors (column 3 and 4 in Table 4.5), suggesting that the effect of calving on inconclusive reactors were uncertain.

In analysis where the definition of recent calving was shortened to be within 30 days (rather than 60) from the reference SICCT test, the results remained significant, however an odds ratio closer to zero (0.66) indicates stronger negative (protective) effect in comparison to the base line model. But estimates from the “week” parameter suggests that this effect may be short lived, with only animals calved within the first week showing statistical significance. Similarly, when case and control samples were restricted to animals with at least one calving (i.e. previous calve > 0), important factors remained in the model as significant. In addition, the positive odds ratio (>1) for “days since previous calving” indicate

Table 4.5: Odds ratios with 95% CI from univariable conditional logistic regression analyses showing the effects of calving related variables on the risk of positive SICCT test result with different case and control definitions and sample sizes

Explanatory variables	(IR + R) vs N	IR vs N	R vs IR	Recent calving (within 30 days)	At least one previous calving
Recent calving (within 60 days)					
No	1	1	1	1	1
Yes	0.81*(0.7, 0.93)	0.9 (0.75, 1.08)	0.88 (0.54, 1.45)	0.66*(0.5, 0.87)	0.71***(0.6, 0.9)
Week (since previous calving)					
Never calved	1	1	1	1	1
or > 8 weeks					
6-8 weeks	0.9 (0.71, 1.13)	1.05 (0.78, 1.42)	0.57 (0.23, 1.42)	0.66 ^d (0.4, 1.1)	0.7 (0.48, 1.02)
4-6 weeks	0.83 (0.64, 1.09)	0.85 (0.59, 1.22)	1.62 (0.7, 3.78)	0.61 ^c (0.36, 1.03)	0.73 (0.5, 1.08)
2-4 weeks	0.76*(0.6, 0.99)	0.81 (0.56, 1.18)	0.68 (0.2, 2.33)	0.86 ^b (0.51, 1.43)	0.78 (0.53, 1.15)
0-2 weeks	0.75*(0.6, 0.98)	0.83 (0.57, 1.19)	0.83 (0.29, 2.35)	0.54*^a (0.3, 0.95)	0.61*(0.4, 0.93)
Days (since previous calving)					
Never calved	1	1	1	1	NA
1-100 days	0.93 (0.81, 1.08)	0.95 (0.78, 1.16)	1.06 (0.64, 1.78)	0.86 (0.7, 1.06)	1
> 100 days	1.16*(1.03, 1.32)	1.08 (0.91, 1.28)	1.02 (0.67, 1.56)	1.22*(1.03, 1.45)	1.41*(1.2, 1.68)

Statistics are from all sensitivity analysis, variable “previous calving” was neglected, because all categories were insignificant in all model output.
Significance levels: *p < 0.05; **p < 0.01; ***p < 0.001
^a recent calving between 0-1 week (“week” variable was redefined to represent weekly bins under recent calving within 30 days)
^b recent calving between 1-2 weeks. ^c recent calving between 2-3 weeks. ^d recent calving between 3-4 weeks.

that the further away the test is from previous calving the more likely the animal will react to the subsequent SICCT test.

Sensitivity analyses were also conducted on the case and control samples from gamma-interferon test, the results were not too dissimilar to the original study. Recent calving, when changed to be within 30 days, was statistically significant (odds ratio = 0.49, p-value < 0.001) and “week” parameter was significant for the first 3 weeks with an increasing odds ratio over the first four weeks post-calving. Finally, analysis of samples with at least one calving prior to the test, showed that both variables were again significant factors with similar estimates to the original model outcome.

4.4 Discussion

Two published studies have addressed the effect of pregnancy and parturition on the cellular immune responses to mycobacterial antigens. Kerr *et al.* (1946) detected a marked depression of sensitivity to bovine tuberculin in bTB reactors in the first 14 days after calving (compared with test results post-inoculation and pre-calving), but the sensitivity returned to normal pre-calving levels 4-6 weeks after calving. However, results from this study can be questioned as reduction in immune responses to *M. bovis* antigens have previously been reported following repeated SICCT testing (Coad *et al.*, 2010). In the second study, Buddle *et al.* (1994) conducted a *Mycobacterium bovis* infection study on experimentally inoculated cattle and examined the effect of pregnancy and parturition on the immune response as part of the study objective. The study concluded that pregnancy did not appear to affect the susceptibility to *M. bovis* infection, and immune response (measured by the absolute difference in skin thickness) of the cattle in the pregnant group at the end of the study were not too dissimilar to those

in non-pregnant group. However, from the first test after calving, the gamma-interferon responses were low compared with the responses prior to calving, and after a further 2-4 weeks, the gamma-interferon responses had returned to pre-calving values. Unfortunately, during that study, the SICCT test was not applied to the pregnant group prior to calving, and therefore direct immune response of the SICCT test immediately before and post-calving cannot be compared.

In contrast, the current case-control study identified calving related factors that significantly impact the response, directly related to the outcome (positive or negative) for the standard SICCT test and gamma-interferon blood test, both commonly used as diagnostic tool for bTB detection in cattle in the UK. Factors such as animal age, breed and farm locations have been shown as confounding factors to bTB incidence in many previous epidemiological studies (Skuce *et al.*, 2011; Brooks-Pollock *et al.*, 2013; Alvarez *et al.*, 2014; Lahuerta-Marin *et al.*, 2016), these were controlled for in the current analysis via a matched case-control design. Some similarities and some differences in the factors associated with SICCT response versus gamma-interferon reactions were revealed. Firstly, non-recently calved animals (more than 60 days) were compared with recently calved animals (within 60 days). The latter animals are known to be associated with the immune response and hence the outcome of the diagnostic test (from experimental study by Buddle *et al.* (1994)). The estimated odds ratio from conditional logistic regression analyses indicate that recent calving within 60 days prior to test was a significant factor that was negatively associated with positive response in both type of tests. In particular, when comparing the estimated odds ratios in face value, the magnitude of the effect appeared to be stronger during periods immediately post-calving (within 14 days) for gamma-interferon test (OR = 0.47, 95%CI: 0.24 – 0.91) compared to the SICCT test (OR = 0.66, 95%CI: 0.45 – 0.99), even though their respective 95% CI largely overlap. Moreover, the estimated odds ratio for the “week” variable revealed that the adverse effect of

recent calving on test positivity declines over time (with increasing odds ratio towards 1), and was non-significant between 2-6 weeks post-calving for SICCT and beyond 4 weeks for gamma-interferon test. Though this decreasing trend seems to be sharper under the gamma-interferon test with a change of sign in the odds ratio estimate for the latter category in “week”, while the associated odds of recent calving under SICCT test remained negative and significant even between 6-8 weeks post-calving. Direct comparison in the reduction for test sensitivity between SICCT and gamma-interferon test during the post-parturient period has not been previously investigated, but this temporary reduction in response to the test outcome (reactor rate) provides an interesting parallel with results from early experimental studies by Buddle *et al.* (1994) and Kerr *et al.* (1946) on *Mycobacterium bovis* infection, and supports the general results of this study. However, a limitation in studies with experimentally infected animal is that the immune responses may be more substantial early after infection, as demonstrated by a study where cattle were artificially inoculated with different doses of *M. bovis* (Schiller *et al.*, 2009). This can potentially limit the ability to demonstrate subtle effects that may have been achieved with lower responses commonly observed in naturally infected field cases (Schiller *et al.*, 2010b). This may offer a plausible explanation that the non-significance for several categories in the risk factor (variable “week”) could be due to existing, but subtle effects, of recent calving that is not completely obvious in the selected observations.

Animals with no previous calving (i.e. not used for breeding purposes) may have different herd management practices that could affect the odds of a positive test. When data were restricted to animals with at least one previous calving, results from conditional logistic regression model showed that the magnitude of odds ratios for positive responses under SICCT and gamma-interferon test were not significantly different in comparison to the baseline model. This was largely because “animal age” was a confounding factor that has been controlled for in

the matched study design, and number of previous calving (i.e. parity) is a proxy for age, so results are not expected to be substantially different between the two models even with reduced sample size (i.e. reduced the power to detect an effect) after removal of none calved animals.

Similarly, the decreased odds of testing positive was also tested in different case and control populations (i.e. when inclusive reactors were combined with reactor samples, or when the threshold of recent calving was re-defined to 30 days rather than 60), the results remained significant and qualitatively similar on re-analysis of the data under both types of tests. However, when inconclusive reactors were compared separately with test negatives and test positives respectively, all calving related factors were also inconclusive (i.e. non-significant), suggesting that the effect of calving on inconclusive reactors is uncertain. Little information is known as to why inconclusive reactors showed less reaction compared to reactors, and whether or not they are truly diseased (most likely to contain both true and false positives). But any potential factors (such as recent calving) for the cause may be over shadowed by the poor test sensitivity. There are studies showing some evidence that herds in Scotland purchasing cattle from other herds with unconfirmed IRs (i.e. IR that never tested positive in subsequent re-tests) were at increased risk of having inconclusive test results, which highlights the difficulty in determining the true disease status of herds with unconfirmed inconclusive reactors (Gates *et al.*, 2013). While other studies are lending support to the evidence that IR has increased future risks of bTB (Clegg *et al.*, 2011b,c), however, generally speaking, immune parameters are known to vary, and hence may reduce the likelihood of detecting a significant effect in different samples. Further investigation is needed to determine the potential causes of non-reactivity for inconclusive reactors.

As expected, the number of previous calvings were not significant under both

SICCT and gamma-interferon analysis, this may be due to the fact that previous calving is associated with animal age, the older the animal, the more calvings would be expected. Furthermore, since animal age was one of the confounding factors that was already be controlled for, and previous calving was correlated with age, the variable was non-significant in the conditional logistic model.

One of the difficulties with a study based on an imperfect diagnostic test, such as the tuberculin test and the gamma-interferon, is the possibility of misclassification of the outcome status. In the analysis, an animal was only regarded as a case if tuberculosis was confirmed by the detection of tuberculous lesions at slaughter or culture positive in one or more of the animals from the same herd. It was not possible to confirm the true status of the control animals, thus giving rise to the possibility that some of these could have been false negatives, although the large sample size and prevalence of the disease makes it less likely. Factors that may result in false negative reactions to the tuberculin and gamma interferon test have been reviewed by Monaghan and others (Monaghan *et al.*, 1994; Costello *et al.*, 1997). They include low sensitivity during early clinical course of infection, desensitisation following a tuberculin test, variation between observers and depressed immune response to bTB antigen caused by physiological stress such as change of environment, high levels of movements prior to testing, pregnancy and parturition (the working hypothesis under the current study). Although it was extremely difficult to distinguish between these influential factors as to why recently calved animals appear to show less response towards the bTB diagnostic test, the possibility of an immunosuppression effect during the early post-partum period cannot be ruled out. In general, based on data from 2002-2012, approximately 7% of UK cattle population were tested during this at-risk period, and therefore caution needs to be taken when interpreting test results of recently calved animals (i.e. less than 4 weeks post-partum), particularly in situations where there was reasons to suspect bTB in the herd for other reasons.

Other literature have suggested that the accumulation of antibodies in the colostrum, prior to, and immediately after, calving, could explain why infected cows three weeks pre-partum to three to six weeks post-partum, tend to show a decreased sensitivity to the tuberculin test (Coetzer and Tustin, 2004). Given the nature and type of field data available on bTB tests, the current study focused only on the effect after calving. Regulations under the test-and-slaughter control policy require all identified reactors to be slaughtered shortly after testing positive (Defra, 2008a), therefore there is a lack of data to explore the effect pre-calving (i.e. reactor animals pre-parturient would be slaughtered prior to giving birth). Despite limited clinical and epidemiological evidence, New Zealand, with a successful bTB control policy (Sinclair *et al.*, 2016), introduced restrictions to avoid testing animals 3 weeks either side of calving to avoid potential miss diagnosis (Vetent, 2015). Government advisors cite hormones in late pregnancy/early lactation as limiting factors that can potentially affect the bTB test (Vetent, 2015). Other limitations in the present study include the use of the CTS database to determine recent calving history. Calves that are stillborn or die very shortly after birth are not recorded in the CTS, which can lead to an underestimation of calving events (Gates *et al.*, 2013).

Recent studies suggest that physiological stress can play a significant role in the complex interplay between susceptibility, host immunity and the pathogen (reviewed by Verbrugghe *et al.* (2012); Skuce *et al.* (2011)). The study stated that stress hormones could influence the macrophage-pathogen interaction and probably affect the outcome of mycobacterial infections. While other authors suggested that pregnancy could potentially increase the susceptibility to mycobacterial infections (Griffin, 1989; Wolfe *et al.*, 2009; Verbrugghe *et al.*, 2012). No direct association between pregnancy and susceptibility to infection/disease was reported in the experiment by Buddle *et al.* (1994), although it is well documented that peri-parturient immune-suppression in dairy cows, which may

be linked to other deficiencies (Kehrli *et al.*, 1989; Burton *et al.*, 2003), might be the causal influence. The potential link between periparturient immune-suppression and mastitis susceptibility in dairy cows was investigated in the USA (Burton *et al.*, 2003) where experimental and field evidence suggested that systemic and local (mammary) immune responses were deficient around parturition, supporting the logical hypothesis that immune deficiency was behind the heightened susceptibility observed in peri-parturient cows. This provides additional evidence to suggest that the observed reduction in test positivity of recent calved animals based on SICCT and gamma-interferon test results in the current study was related to the performance of the test rather than reduced susceptibility to the disease. Although the reduced likelihood (odds) of testing positive can not be proved to directly relate to reduced immune response using data from the present study alone, the significantly low number of test positives for bTB infection in recently calved animals implies that a depressed immune response could be one major potential cause for the unresponsiveness observed in this sub-population.

It must also be acknowledged that there were several other risk factors which may cause the immune response to decrease, further studies will be conducted to explore this in detail (next chapter analysis on false negatives). Findings from experimental studies directly investigating the immune response to bTB antigens (Kerr *et al.*, 1946; Buddle *et al.*, 1994), or other bTB diagnostic tests (Monaghan *et al.*, 1994; Skuce *et al.*, 2011; Costello *et al.*, 1997) would support the concept of false negatives due to depressed immune response caused by pregnancy, lactation and parturition (i.e. stress-related events).

Chapter 5

Risk factors for missing infection from the SICCT test for bovine tuberculosis in cattle related to physiological stress

5.1 Introduction

The complex epidemiology of bovine tuberculosis (bTB) combined with the lack of a gold standard diagnostic test means that it is extremely difficult to detect and eradicate the disease on a regional or national scale (Durr and Hewinson, 2000; Conlan *et al.*, 2012). As a result, bTB is a persistent economic and veterinary problem in cattle herds in the UK. Despite an intensive and costly test-and-slaughter control program, the rate of herd breakdowns has progressively increased over the past 25 years (Defra, 2014a; Gilbert *et al.*, 2005). Studies

have shown that 38% of herds in the Britain that clear movement restrictions experience a recurrent incident within 24 month (Karolemeas *et al.*, 2011). In Northern Ireland, a slightly higher proportion of herds (42%) suffered further breakdowns (Doyle *et al.*, 2014), while in Republic of Ireland, approximately 35% of herds had a subsequent breakdown following derestriction (Gallagher *et al.*, 2013). This high rate of recurrence suggests that infection may be persisting within herds in the face of repeated testing (Conlan *et al.*, 2012).

The single intra-dermal comparative cervical tuberculin (SICCT) test is used as the primary screening test for bTB in the UK (de la Rua-Domenech *et al.*, 2006). Despite studies suggesting the sensitivity and specificity of the SICCT can be as high as 80% (ranging from 50-90%) and 99.9% respectively at standard interpretation (Monaghan *et al.*, 1994; Downs *et al.*, 2011; Clegg *et al.*, 2011a; Hartnack and Torgerson, 2012), the performance and efficacy of the test is dependent upon the animal population studied and can be difficult to quantify and generalise over the whole cattle population (Strain *et al.*, 2011). More importantly, the SICCT test is essentially designed to detect an immune response to injected bovine tuberculin rather than the signs of disease (Bovine TB Advisory Group, 2009). As a result, *post-mortem* examinations of animal carcasses and culturing of tissues from visible lesions for the causative bacterium are used as confirmation of infection (Defra, 2008a). However, the success of tissue culture mainly depends on the presence or absence of visible lesions in the carcass and samples submitted to the laboratory and is closely linked to the stage of *M. bovis* infection (Goodchild and Clifton-Hadley, 2006). Due to the chronic nature of the disease, it can take between a few weeks to several years for the development of visible pathology typical of bTB (most commonly found in the lymph nodes of the respiratory and gastrointestinal tract reflecting the route of infection in an infected animal) (OIE, 2009). Though experimental studies have shown that microscopic lesions consistent with tuberculosis can develop within 15 – 28 days

from initial inoculation. However, gross visible lesions are more likely to be seen from 42 – 60 days after inoculation (Palmer *et al.*, 2007).

Currently in the UK, all SICCT reactors (i.e. test positive cattle) are removed from the herd, slaughtered and have their carcasses inspected for the presence of visible lesions, but not all cases are cultured to save cost (generally, once culture is confirmed on at least one animal in a new breakdown herd, the rest are ignored and assumed to be positive). Though only direct culturing of *M. bovis* or other molecular methods can provide definitive evidence of infection (Shitaye *et al.*, 2006), animals with gross pathology lesions (i.e. not 100% confirmation of *M. bovis* infection) should also be considered as potential transmitters that pose continuous or intermittent threat to the disease security of the herd (Kao *et al.*, 2007). Particularly in developed countries with established disease surveillance and advanced control programmes, a possible SICCT reactor combined with visible lesions provide strong evidence of infection.

Traditionally, assessment of bTB diagnostic test performance has focused on identifying as many infected animals as possible. However, in reality, not all animals infected with bTB will be infectious. Some, such as those that are immuno-compromised or in the later stages of infection (potential ‘super-spreaders’ that are more likely than average to infect others (Kao *et al.*, 2007; O’Hare *et al.*, 2014)) may well be responsible for a disproportionately high proportion of disease transmission (Strain *et al.*, 2011). Therefore, infected animals missed by the SICCT (i.e. false negatives) may have a greater risk of contributing to the silent spread of the disease, and pose a significant challenge to disease control and eradication (Conlan *et al.*, 2012).

Current literature suggests that the risk of false negatives from the SICCT test under field conditions is dependent on a range of variables, such as the diligence of the tester (Defra, 2014a) in adhering to the correct testing procedure, the

within-herd prevalence of cattle sensitised to other non-tubercular mycobacteria (NTMs) from the environment (Shitaye *et al.*, 2009), and factors that may alter the immune response (to tuberculin) of individual animals. For example, factors such as nutritional level, previous exposure to bovine tuberculin via repetitive testing, concurrent infections and stages of infection can all potentially affect the immune response to SICCT test (Costello *et al.*, 1997; Clegg *et al.*, 2011a; Claridge *et al.*, 2012). In addition, a recent case-control analysis on Northern Ireland cattle herds by Lahuerta-Marin *et al.* (2016) identified age-class, herd production type, farm location and seasonality as potential risk factors for failure of *ante-mortem* tests to detect all confirmed infections.

There is general consensus (though lack of direct biological evidence) that physiological stress may also alter the immune response to the SICCT (Buddle *et al.*, 1994; Mallard *et al.*, 1998; de la Rua-Domenech *et al.*, 2006; Gates *et al.*, 2013) and therefore contribute to the heightened risk of false negative results. The stress of handling, testing, movement and calving may play a key role in the disease diagnostic performances, but data are sparse and few analyses have been done to examine their effects (Skuce *et al.*, 2011; Verbrugghe *et al.*, 2011). The objective of this study was to explore whether certain life history events that may cause “stress” were associated with the risk of being false negatives and thereby compromise the underlying test sensitivity.

It is shown from related studies in the previous chapter for cattle farms in bTB endemic regions of Great Britain that the responsiveness (i.e. positive or negative outcome) of SICCT and gamma-interferon blood assay can potentially be influenced by calving events and recent parturition (Chapter 4). However, animals may be non-responsive to the diagnostic test due to absence of disease, the possible protective effect of calving may be in relation to contracting the disease and therefore not a real immune-suppression effect from subsequent testing. In this

chapter, case-control studies were carried out using only “infected” individuals to explore stress-related events (i.e. previous SICCT test history, recent movements and calving) as potential risk factors for false negative SICCT test results. The analysis was based on all animals that developed pathology where either TB-like lesions and/or bacterial culture were detected. A SICCT that was conducted a relatively short time before the animal was sent to slaughter should therefore be either a true positive or false negative (given the imperfect nature of the test). Therefore this is used to define the case and control groups for comparison:

- “cases” were defined as SICCT negatives tested within 60 days prior to slaughter, and presented with visible lesion and/or positive *M. bovis* culture at *post-mortem*.
- “controls” were test positives which had successful *M. bovis* culture following *post-mortem* at slaughter.

A list of stress-related risk factors including movements and testing along with individual animal and herd characteristic were included in two logistic regression models (matched and unmatched design) to explore their effect on the false negative rate of the standard SICCT test (i.e. probability of missing infection).

5.2 Materials and methods

5.2.1 Datasets

As part of bTB control and surveillance system, data collection on bTB test has been ongoing in Great Britain since the mid 1990s. Records of all bTB

testing results and breakdown history in all cattle herds in Great Britain (GB) are contained in the Sam’s IT system, which is collated and managed by the Animal Health and Veterinary Laboratories Agency (AHVLA). It consists of bTB test history, herd breakdown details and individual reactors information including *post-mortem* results and culture. Cattle demographic and movement information are contained within the Cattle Tracing System (CTS), run by the British Cattle Movement Service (BCMS). During the 2001 Foot and Mouth outbreak in the UK, bTB testing substantially reduced (Carrique-Mas *et al.*, 2008), therefore data from the Sam’s IT system covering bTB tests between 2002 and 2012 were extracted, and CTS data from the same period were used for the analysis. All datasets were extracted using PostgreSQL with data analysis and statistical modelling carried out in R.

5.2.2 Data manipulation

A series of data extractions, filtering and matching procedures were carried out based on the Sam’s IT system and CTS extracts (from 2002 - 2012) to deduce the case and control groups. The process is summarised in Figure 5.1 and in described in details as follows:

1. All animals born after 1st Jan 2002, which were identified with gross visible lesions (VL) at slaughter or successful culture of *M. bovis* at laboratory were selected from the “Animal” table in the Sam’s IT system. These animals were deemed to be “truly” infected with bTB.
2. Individual animal characteristics and historical residency locations were extracted from the “livestock locations” table in the CTS database by cross-referencing the animal’s ear-tag number in the Sam’s IT system records.

3. At the individual animal level, previous SICCT tests for the duration of residence in each farm holdings were inferred from the “test” table in the Sam’s IT system using “start date” and “end date” for each residency record identified in CTS (note, only results where the ‘whole herd’ was tested (rather than ‘individual animal’ tests) were included in the selection criteria, see table 1 for list of tests classified as whole herd test under Defra’s eligibility guidelines).
4. From the list of cattle that were present during a test, Defra’s eligibility guidelines were used to determine which of the cattle had been eligible for the test and assumed that all eligible animals present were tested.
5. Finally, complete herd breakdown histories since 2002 for all destination farms (that received animals) were extracted from the “breakdown” table in the Sam’s IT system.

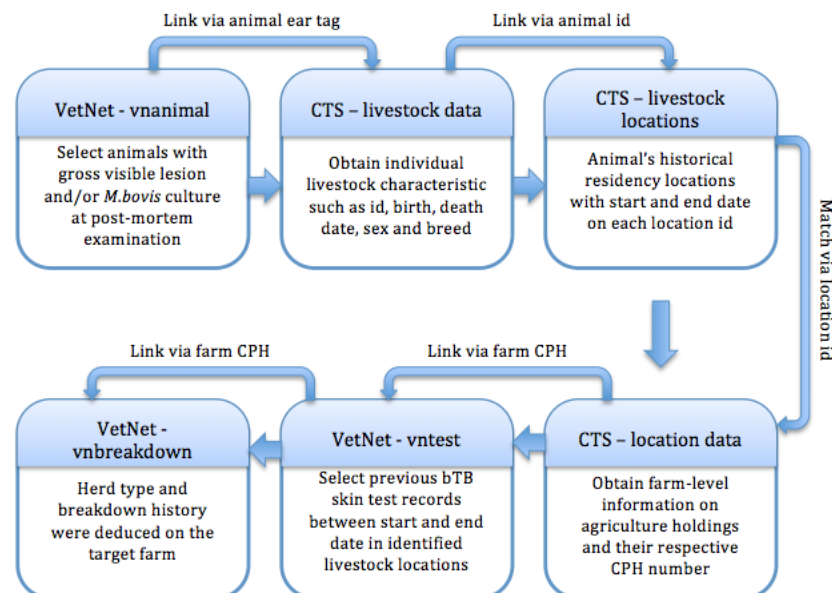


Figure 5.1: Flow diagram showing the process of data extraction and linkage between the Sam’s IT system and the CTS database

Only records where the “whole herd” is tested (as opposed to individual animal tests) were included in the selection criteria over the 10 year period, see Table 5.1 for list of specific tests classified as whole herd test under Defra’s eligibility guidelines. By filtering animals without positive *post-mortem* diagnostic (i.e. VL or culturing), all case and control animals were assumed to be truly infected with the disease prior to slaughter.

From here onwards, the term “SICCT tests” refers to whole herd SICCT tests outlined in Table 5.1, unless stated otherwise. Other non-frequent and minority whole herd tests (not listed in Table 5.1) such as: New Herd test (VE-NH1/NH2), Approved Segregated Group test (VE-ASG) and various forms of Check tests (VE-CT and VE-HS1/HS2) were not included in the analysis due to the ambiguity of test cohort (unable to reliably determine which animals were tested).

Table 5.1: SICCT test code and description. All tests listed were conducted as a whole herd with certain exception criteria outlined in the “Test eligibility” column. (Information obtained from AHVLA TR15(E) form)

Type of test / cycle	Test code	Test eligibility
Whole Herd Test (Routinely every 12 months or 2 yearly)	VE-WHT VE-WHT2	All bovines except calves under 6 weeks of age
Routine Herd Test (at 24, 36 or 48-month interval)	VE-RHT24 VE-RHT36 VE-RHT48	All breeding bulls over 12 months of age and females which have calved. Breeding bovines purchased since last herd test over 6 weeks of age
Short Interval Test (60 days after removal of the last reactor whilst the herd is under movement restriction)	VE-SI	All bovines except calves under 6 weeks of age, this exemption exclude herds with known risk of infection
Six Month Test (6 months from the date of clear SI test)	VE-6M	Non-recent move more than 60 days ago
Twelve Month Test (12 months after 6M tests. or 6-12 months after last SI test in unconfirmed breakdowns)	VE-12M	All bovines except calves under 6 weeks of age, this exemption exclude herds with known risk of infection
TB Unit Test (Test every 90 days on approved finishing units)	VE-TBU VE-90D (days)	All bovines
Contiguous Herd Test (Carried out on herds contiguous to breakdown herds outside their regular test frequency)	VE-CON VE-CON6 VE-CON12 (months)	All bovines except calves under 6 weeks of age, this exemption exclude herds with known risk of infection

5.2.2.1 Selection of case and control animals

SICCT test results were classified into three main categories under the Sam's IT system structure. Reactor cattle from SICCT test, were identified as "R" (this means that the immune response for bovine tuberculin was at least 4mm or greater than avian reaction under the standard SICCT interpretation), and inconclusive reactors were denoted as "IR" (2-4 mm difference between bovine and avian reaction), while slaughterhouse suspect cases with TB-like lesions from regular abattoir examination were recorded as "SL". In addition, based on risk assessment and disease status of the herd, there were two standards of interpretation for SICCT test results; "standard" and "severe". Under the "severe" interpretation, 2-4mm threshold is used for animals to be classed as reactors to enhance the sensitivity of the test in high risk suspect herds. For the purpose of analysis in this chapter, only animals under "R" by standard interpretation and "SL" that fulfilled the following criteria were selected as case and control group.

A case animal was a false negative identified by meeting the following criteria:

1. Slaughterhouse suspect case (i.e. sent to slaughter not because of a positive test but identified with gross visible lesions at regular *post-mortem*). And with evidence of gross pathology and/or positive culture results (i.e. deemed to be infected at time of slaughter).
2. Eligible for WH SICCT test and tested negative within the 60 days prior to slaughter (assume animal were infected at time of test). The animal may have been gamma-interferon (IFN) test positive but was SICCT tested negative within 60 days prior to slaughter (minimum period for gross lesion development).

3. No previous inconclusive test results (other than the most recent IFN test prior to slaughter).

The control group consisted of:

1. All test positives from SICCT test (under standard interpretation).
2. Slaughtered within 60 days of test
3. *Post-mortem* positive (infection confirmed through identification of VL or culturing).
4. No previous inconclusive test results.

5.2.2.2 Inferring the reference SICCT test

The SICCT test that determines the cases (i.e. SICCT test negative) and controls (i.e. SICCT test positive), according to the selection criteria, are referred to as the reference SICCT test from here onwards. This is the last SICCT test occasion immediately prior to slaughter. For animals in the control group, the reference test can be easily obtained directly from the “Animal” table in the Sam’s IT system (i.e. it is the date which the animals were SICCT tested positive). However, in general, there is no historic information in the Sam’s IT system about cattle that tested negative to SICCT prior to 2013 (Brooks-Pollock *et al.*, 2013). In order to calculate the most recent SICCT test for each animal in the case definition (i.e. false negatives), SICCT test history for each individual animal were reconstructed by combining herd-level testing data from the Sam’s IT system with animal-level data from the CTS database (see Figure 5.1 for schema of data linkage). The following sequential steps were carried out to determine the reference SICCT test occasion for the case group.

1. By definition, “SL” cases and gamma interferon reactors had never been SICCT tested positive, therefore an eligible SICCT test 60 days prior to the “death date” of the animal must have been negative.
2. SICCT test histories were obtained on all residency locations for each eligible and present animal in the case and control groups (follow Figure 5.1 for data matching procedure between the CTS “location data” and “vntest” in the Sam’s IT system).
3. The most recent eligible SICCT test within 60 days before the animal’s death date was chosen as the reference test date.
4. All animals not eligible for SICCT test within 60 days prior to slaughter were removed.
5. The type of reference SICCT test (e.g. RHT, SI etc ...), test date and the unique test id were extracted from the “vntest” table in the Sam’s IT system.

Diagnostic test outcome, individual animal characteristics, historical movement records and previous SICCT test pattern were inferred and calculated in relation to the reference SICCT test occasion. The test outcome was codified according to the case-control definition, 0 = SICCT test positive (control), and 1 = SICCT test negative (case) conditional on being culture or lesion positive at slaughter. This is the response variable of interest and distinguishes between case and control. It is also used as the outcome variable in the statistical analysis.

5.2.2.3 Calculating stress-related risk factors

There are two main types of stressors examined in this analysis, potential stress caused by animal movements and previous SICCT test experience (including exposure to bTB antigens and potential physical stress caused by frequent and multiple testing). The respective stress-related risk factors can be further divided based on frequency and proximity from the reference SICCT test. While the total number of animal movements and previous SICCT test were aggregated from birth, the time since recent SICCT test and recent movement prior to the reference test were calculated and categorised into different risk groups (shown in Table 5.2).

The movement history for each animal identified from the case and control selection criteria were traced using the “livestock location” table in CTS by matching on the animal ear tag number. The total number of movements (other than final movement to abattoir) since birth was aggregated for each animal. Each movement record contained the start and end dates on the respective premises. The most recent movement record was then extracted along with the pre-determined reference test date to calculate the time interval (in days) since the last movement (i.e. reference test date – latest start date).

Previous calving activities for all female cattle used for breeding were obtained from the “livestock relationship” table in the CTS. The records contain birth date and identification number for each calf linked by the “id number” of its dam. The dam “id number” was matched for all case and control samples and the total number of calvings were aggregated from records in the “livestock relationship” table. Pre-determined reference SICCT test for each animals were then matched, and the time interval since previous (latest) calving were calculated

Table 5.2: Stress-related risk factors calculated from birth to the point of the reference SICCT test for each case and control animals

Stress-related risk factors	Values	Description
Total number of movements	Numeric (0–31)	Aggregated movement frequency from birth to the reference SICCT test ¹
Time since recent move	0	Home-bred animal that never moved away
	1	Non-recent move more than 60 days ago
	2	Recently moved onto the farm within 60 days prior to the reference SICCT test
Cumulative number of SICCT tests	Numeric (0–35)	Aggregated SICCT test frequency from birth to the reference SICCT test (using the unique test id)
Average annual SICCT test frequency	Numeric (0–7)	Average number of SICCT test per year based on animals's life span
Time interval since previous SICCT test	0	Never SICCT tested apart from the reference test
	1	Previous SICCT test was more than 120 days ago
	2	Previous SICCT test is between 60–120 days prior to the reference test
	3	SICCT test is within 60 days prior to the reference test
Cumulative number of calvings	Numeric (0–10)	Aggregated calving occasions within animals's life span
Time interval since previous calving	0	Calving more than 30 days prior to test
	1	Recent calving within 30 days

¹ Animal moving off one premise and onto another is counted as 1 movement, consequently, an animal traded through a market counts as 2 movements.

by subtracting the reference SICCT test date and the birth date from its latest calve.

Similarly, detailed information was also obtained on previous whole herd tests prior to the reference test for each case and control animal. The complete herd-level SICCT test history were inferred from the “vntest” table in the Sam’s IT system between the start and end dates in each movement record (as obtained from calculation of movement histories). Individual animals were then assessed using Defra’s eligibility guidelines to construct animal-level testing data for each movement record. As well as the total cumulative number of SICCT tests each animal had experienced, the average annual test frequency was calculated based on an animal’s life span to account for potential age heterogeneity (the older the animal the more SICCT tests it is likely to have experienced). The time interval since the previous herd test was calculated by subtracting the latest SICCT test date from the reference test date.

5.2.2.4 Animal-level and herd-level risk factors

The risk factors associated with animal characteristics and herd demographics (herd where reference test was carried out) are listed in Table 5.3. Animal age at the point of the reference SICCT test were calculated and categorised into sub-groups. A previous study by (Brooks-Pollock *et al.*, 2013) suggested that animals aged between 1-3 years had the highest rate of infection (incidents) with *M. bovis*, and consequently these animals were grouped under one category. Younger animals, less than 1 year of age and older animals, more than 3 years make up the remaining categories.

The cumulative number of breakdowns since 2002 was calculated using the “breakdown” table in the Sam’s IT system, where each breakdown episode

was assigned a unique breakdown id, this was aggregated while excluding the breakdown incident associated with the reference SICCT test. A binary categorical variable representing herds with confirmed breakdown records in the last 4 years and herds with no previous breakdown incidences were also created. Information on animal age, breed and sex were obtained from the “livestock data” in the CTS database, and the herd type were extracted from the “herd” table in the Sam’s IT system before being summarised into 5 main herd categories.

Prior to categorisation of the relevant variables described in Table 5.2 and 5.3, a matrix of scatter plots were produced and the associated Pearson’s correlation coefficient were estimated to assess the potential correlations between all combinations of pairs of variables.

Table 5.3: Animal-level and herd-level factors calculated at the point of reference SICCT test for each animal in the case and control samples

Other risk factors	Values	Description
Animal age category	1	0–1 years old at the point of reference test date
	2	1–3 years old at the point of reference test date
	3	> 3 years old
Animal breed	Categorical	The biological breed of the animal including cross breeds)
Animal sex	Male/Female	Sex denoted by M/F (can be both Bull and Dairy)
Herd type	Beef	Main herd types classified under Sam’s IT data definition structure
	Dairy	
	Suckler	
	Finishing/Store Other	
Herd breakdown number	Numeric (0–21)	Total number of breakdown incidents since 2002
Previous breakdown incidents	0	No record of previous breakdown incidence
	1	Confirmed breakdown incidence recorded in the last 4 years

5.2.2.5 Data quality issues

Information about individual cattle and individual herds were recorded differently in the Sam's IT system and the CTS database. CTS use animal id and location id to record animal movement and farm locations, whilst the Sam's IT system refers to animal's ear tag number and farm CPH (county parish holding) code. As a result, a number of observations were excluded from the analysis because the cattle ear-tag or the farm CPH recorded in the Sam's IT system structure could not be identified and matched with records in the CTS database. Data loss also occurred when animals had incomplete movement records, or an inconsistent or invalid death date. The linkage efficiency and data shrinkage for each step of matching between the Sam's IT system and the CTS database are displayed in Figure 5.2.

5.2.3 Statistical analyses

There were two units of observation in this study, herd and animal-level. However, both are associated with test-level outcome where each SICCT test conducted on cattle herds can be uniquely identified using the designated "test id" within the Sam's IT system structure. Hence, diagnostic test result as well as all variables outlined in Tables 5.2 and 5.3 were calculated and extracted based on the unique "test id" linked to the underlying reference SICCT test. The "test id" uniquely identifies different farms as well as different SICCT test occasions within the farm.

As a result of the case and control selection criteria, samples can be selected from the same farm and under the same test occasion over the 10 year period. Due to farm management practices animals from the same farm are more likely

to share similar experience on testing history, movement pattern and calving activities. This means that observations from the same farm are not entirely independent and furthermore, there may be potential variations between different test occasions within the same farm. Therefore, in order to incorporate test and farm-level variability, a mixed effect logistic regression model was carried out to evaluate the effects of stress-related factors (as well as animal and herd level characteristics) on the risk of false negative outcome from the SICCT test.

The response (dependent) variable is binary representing case (1) or control (0) and was assumed to be binomially distributed. Categorical and continuous variables listed in Tables 5.2 and 5.3 were included as independent fixed effects. To account for potential over-dispersion (i.e. extra variation in the data) and observational independence issues arising from animals tested on the same farm and on the same occasion (e.g. not every animal on the same farm is tested at the same time), the unique “test id” was included as a random effect in the model. In another words, the category “test id” consists of two levels of information; 1) unique farm identification, 2) unique testing occasion within each farm location. Due to large number of levels in the random effect (9,387 different “test id”) and the excess of zero-inflated factor variables, Integrated Nested Laplace Approximation (‘INLA’ package in R) was used as an analytical tool to conduct the random effect logistic regression analysis. This method uses Bayesian inference to estimate model parameters and are much more computationally efficient (Rue and Martino, 2009).

Preliminary univariable logistic regression analyses were performed to explore the association between each individual factors and the SICCT test outcome. Variables with 95% credible intervals that does not contain zero were selected for inclusion in the multivariable logistic regression model (inclusion criteria). Components of the final multivariable model were determined by a backwards stepwise

elimination process in which variables that did not meet the inclusion criteria were sequentially removed in turn until all the remaining variables in the model satisfies the inclusion criteria. Forward stepwise selection was then performed adding in each of the eliminated variables in turn and checking for improvements in the model fit to ensure that none of the variables were excluded based on the order elimination. The deviance information criterion (DIC) were used in combination with the inclusion criteria to assist in assessing the “best” model fit under multivariable analyses (lower DIC score indicate preferred model). Since male and beef cattle tend to be managed differently to female or dairy cattle, an interaction between sex-age and breed-age was also evaluated. Other possible interactions of age and sex with the stress variable were included and retained with their main effects if the interaction term improves the final model. For all logistic regression models, the odds ratio (based on the mean posterior estimate) and 95% credible intervals of the selected independent variables associated with the outcome were reported. Only variables with 95% CIs that does not contain 0 were included in the final multivariable model. Model diagnostics were carried out by examining the model residuals to identify potential violation of model assumptions. Variance inflation factors (VIF) were calculated after variable selections in the final multivariable logistic model. This statistic is often used as an indicator on how much of the inflation of the standard error could be caused by collinearity. The cattle movement and bTB testing data were extracted from the CTS and the Sam’s IT system respectively using postgresQL and all statistical analyses were carried out using the INLA package in R (R, 2012).

5.2.3.1 Sensitivity analysis of risk factor on confirmed infection

In practice, there are two broad categories of tests for the diagnosis of tuberculosis in cattle namely direct and indirect tests. Indirect tests identify

infection in live animals using indirect indicators of infection (e.g. immunological markers for reactor definition from the SICCT test). Direct tests are those designed to directly identify the organism in the host animal. Primarily this relates to the *post-mortem* examination of animals and the associated tests used to confirm infection. However, while typical gross pathological changes can be indicative of infection they are not definitive, and a final determination of infection status can only be reached on using confirmatory tests, most notably using bacteriology and/or molecular methods through routine culturing process (Strain *et al.*, 2011). Hence a sensitivity analysis was carried out to restrict case and control samples that only have *Mycobacteria bovis* isolated from routine cultural methods following *post-mortem* diagnostics. The same statistical approach was used to test for associations between each independent variables and the SICCT test outcome on confirmed infections.

5.2.3.2 Sensitivity analysis of minimum cut-off value of lesion development

It was also suggested that cattle experimentally infected with *M. bovis* showed a steady progression through granuloma stages and that by as early as day 42 after initial inoculation, gross pathology can be observed, though only limited necrosis can be identified through visible inspection (Palmer *et al.*, 2007). As a result, a separate sensitivity analysis was conducted where the cases and controls were redefined using a 42 days threshold between the underlying reference SICCT test to the point of slaughter. Specifically, under the same criteria, case animals subject to SICCT test within 42 days prior to slaughter were selected instead of 60 days. Similarly, control animals must be slaughtered within 42 days from the corresponding positive SICCT test. The same data analysis and statistical

methods were performed to examine if results were sensitive to changes in the threshold value.

5.2.4 Subset analysis for calving related events

Analyses to explore the association of calving event on the risk of false negative SICCT test outcome were conducted by restricting case and control samples to female cattle with at least one previous calving event. Consequently, all male cattle and female cattle with no previous recorded calving activities were removed under this analysis. The same statistical methods were used on the resulting dataset to assess the relationship between each independent variable and the SICCT test outcome.

5.3 Results

5.3.1 Case and control samples

The data reduction following each step of matching between the Sam's IT system and the CTS database are displayed in Figure 5.2. The Sam's IT system was the starting point to which the CTS database were linked.

The case and control samples were selected from 82,714 animals that had positive *post-mortem* diagnosis (i.e. VL or culture) and with complete movement and testing histories identified from the period between 2002–2012. This was approximately 25% of all bTB positive or suspected cases in the UK. A total of 2,022 animals fulfilled the case definition, of which more than 70% were "SL"

cases, around 30% were “IFN- γ ” positives (and SICCT negatives) and all of these were included in the analysis. The control group consisted of 14,304 SICCT test positive animals under “standard” interpretation, and they came from confirmed breakdown herds across the UK for the study period (majority of confirmed test positives were from high incidence areas in south-west of England and Wales). In total, the final case and control samples consisted of 16,326 animals and 8,337 unique reference SICCT test instances from 5,881 farms.

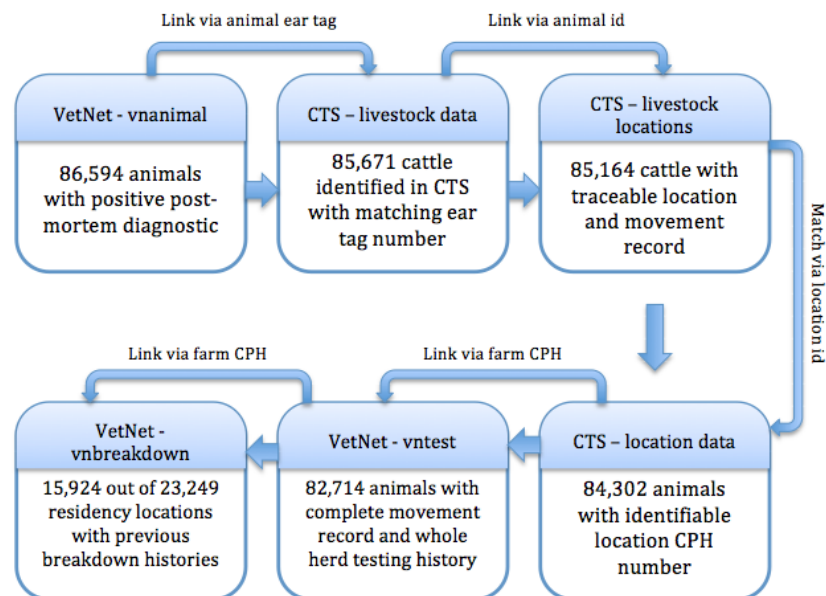


Figure 5.2: Flow diagram showing data shrinkage during each step of matching between the Sam's IT system and the CTS database

Descriptive statistics on the frequency of the reference SICCT test type are presented in Figure 5.3. WHT (29%) and SI (26%) are the most common test type in the study, however a significant proportion of tests were 6-month (6M) and 12-month (12M) tests which were additional examinations following previous clear SI test. By contrast, routine herd tests (RHT, generally conducted in low prevalence areas) and contiguous herd tests (CON), identified the fewest cases and controls.

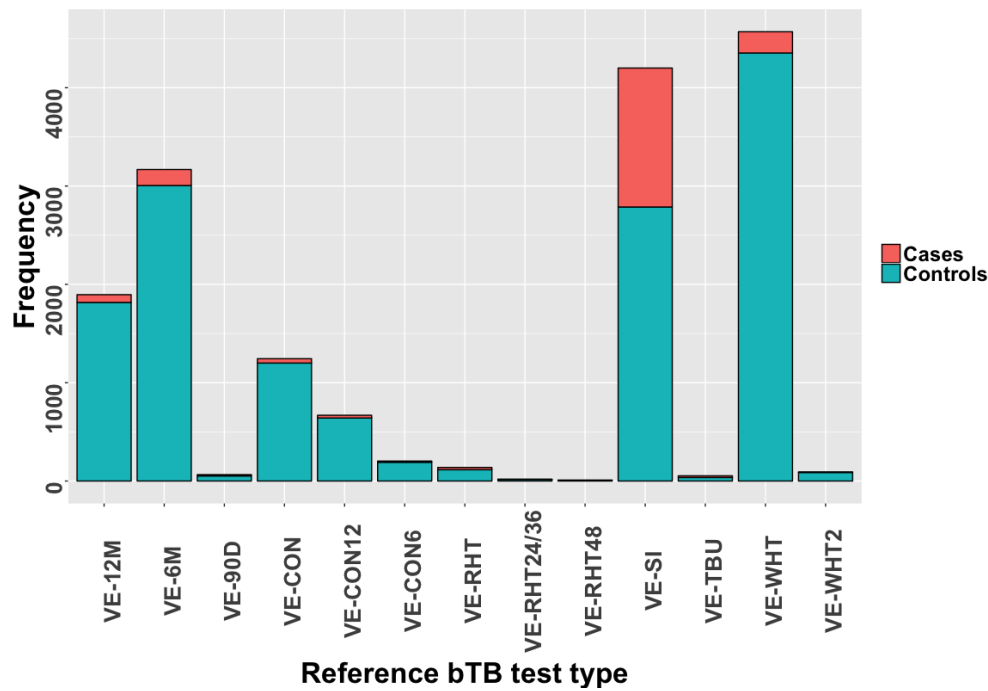


Figure 5.3: Frequency distribution of selected case and control samples by bTB test type outlined in Table 5.1

5.3.2 Descriptive analysis

There are 26 different breeds of cattle in total within the case and control sample. It was not plausible to treat them as separate categories within the analysis due to excess number of variables to fit. Therefore animal breed was generalised into beef and dairy types (cross breeds were treated as beef). The age distribution (in years) for cases and controls stratified by sex and breed is shown in Figure 5.4. More than 70% of the animals were female and beef cattle constituted 57% of the female category, while male dairy represented 4% of the sample. The mean age of cattle identified as “cases” was 3.5 years (median: 2.43, range: 0.5 to 10.55) and 69% were female cattle, whereas the mean age of “controls” was moderately younger at 2.78 years (median: 2.1, range: 0.2 to 10.95) and 73% were female cattle. The age distribution amongst cases and controls and between different sex-breed categories are shown in Figure 5.4.

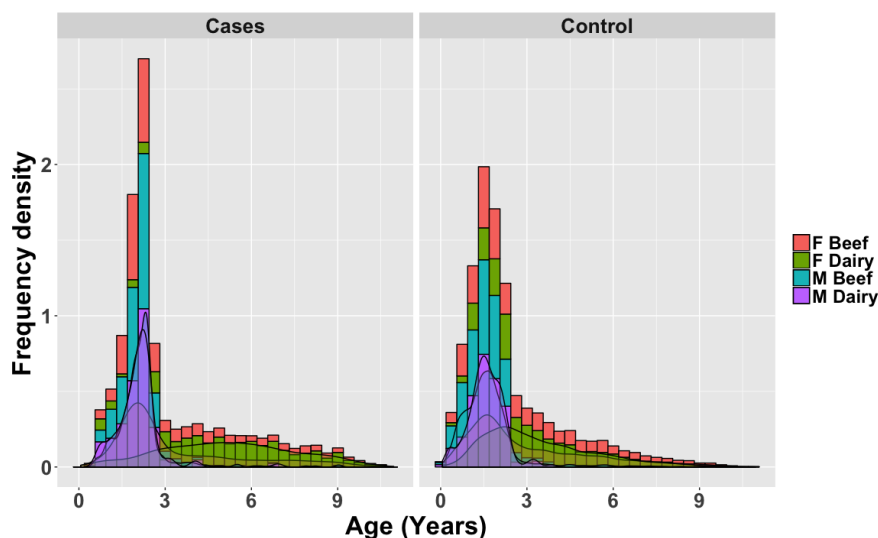


Figure 5.4: Stacked frequency density distribution (displayed as shaded smoothed curve) of cattle demographics and age of the cases and controls

A scatter plot matrix (Figure 5.5) is produced to make pair-wise comparison between each risk factors and examine potential correlations on a linear scale (i.e. prior to categorisation). From Figure 5.5, it can be seen that the average annual test frequency and total number of test showed the largest Pearson's correlation coefficient estimate (0.67), while a positive correlation (0.66) also exist between test numbers and age. The total number of movements was weakly correlated with time since previous moves (0.48), although this may be due to the presence of large numbers of home-bred animals with 0 movements. The scatter plot of time interval since previous test (i.e. plots from second column in Figure 5.5) showed three distinct SICCT test instances at 2, 6 and 12 months. This is directly related to the high number of short interval (60 days), 6 months and annual WHT test identified in the case and control samples (shown in Figure 5.3). Time since previous movement against age shows that animals younger than 3 years were moved more recently prior to the reference test (perhaps due to frequent movement), though more than 60% of the samples were home-bred animals with no movement record. Majority of case and control samples had more than one whole-herd test in the past.

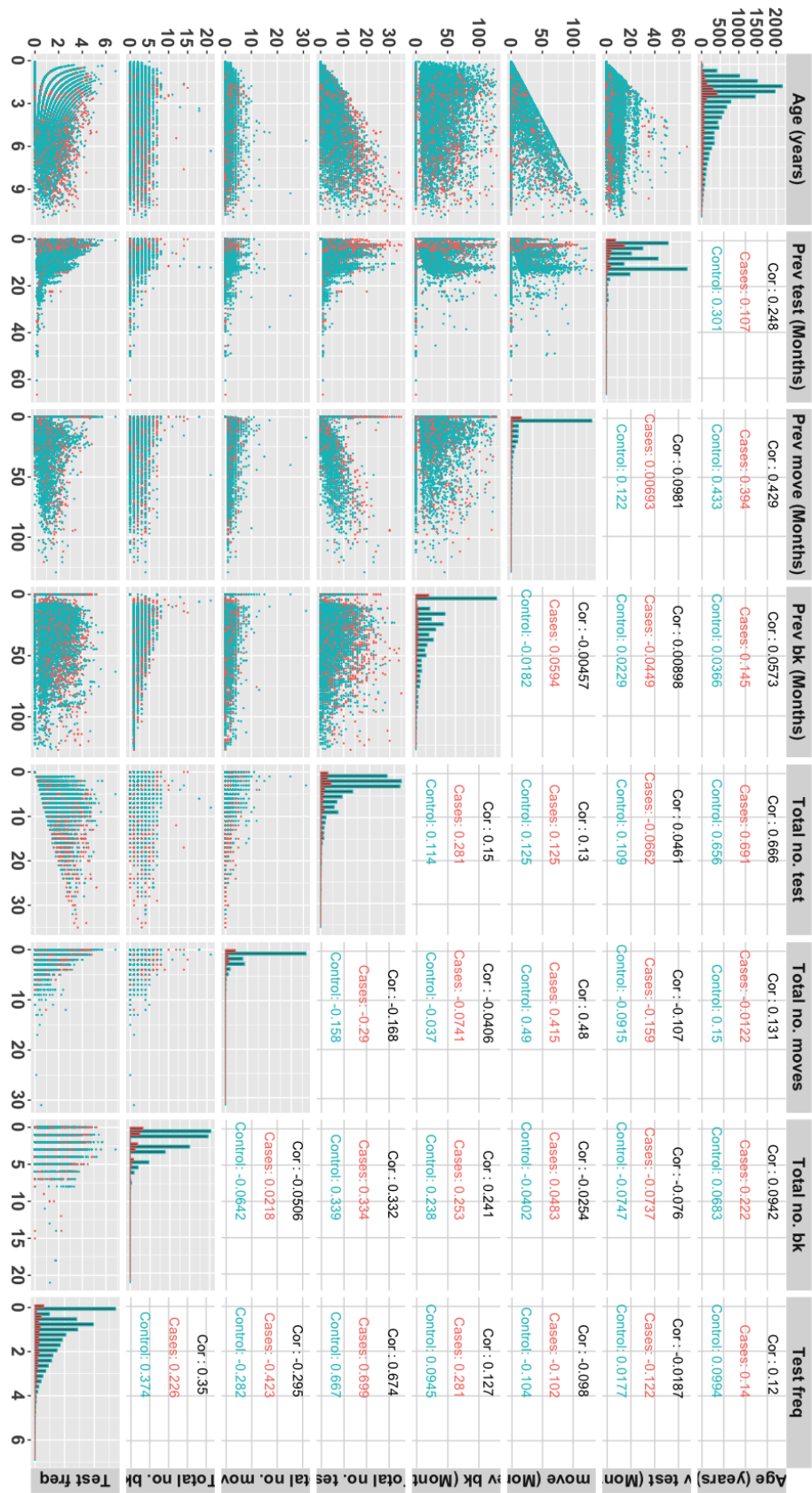


Figure 5.5: Scatter plot and the calculated Pearson's correlation coefficient between every pairwise combinations of risk factors. 1 represent cases (shown in green) and 0 represent controls (shown in red).

5.3.3 Univariable analyses

The estimated odds ratios for categorical risk factors are shown in Table 5.4. Means and standard deviation of continuous risk factors are shown in Table 5.5. Differences in the odds ratio estimates were observed between the case and control animals in all factors examined under univariable logistic regression models (shown in Table 5.4). More importantly, categories for stress-related events such as previous SICCT test and recent movement have estimated 95% credible intervals not containing zero and positive odds ratios that measures the magnitude of association with missing infections at SICCT test.

There was a significant increasing trend in the odds of false negative SICCT outcome when tested closer in relation to the previous test. Successive tests more than 120 days apart were 0.68 (95%CI: 0.58 - 0.79) times less likely to be false negative in SICCT testing compared to animals with no previous tests. The odds ratio increased dramatically when repeated testing occurs between 60 - 120 days (5.34, 95%CI: 4.57 - 6.26) and 0 - 60 days (7.30: 95%CI: 5.65 - 9.42) respectively. Similar trends were observed in movement activity, the odds of mis-diagnosing an infection is highest if the test was conducted closer to an animal movement date. In particular, SICCT test conducted within 60 days of between-herd movement is more likely to be a false negative (2.37, 95%CI: 1.60 - 3.44) compared to home-bred animals with no movements or movements more than 60 days prior to test (1.43, 95%CI: 1.29 - 1.58). Furthermore, on animal-level characteristics, the estimated odds ratio suggest that animals in older age category were more likely to be false negatives under standard SICCT compared with younger animals and male or dairy animals has higher risk for missing infection compared with female and beef categories respectively. Regarding herd-level factors, using beef herd as the baseline type, dairy, finishing/store and other types were more likely to test false negative while animals from suckler herds were 0.77 times likely to be false

negative. The risk for animals from herds with at least one previous confirmed breakdown incidence was 0.91 times in comparison to herds with no recorded breakdowns in the previous 4 years.

Continuous factors including SICCT test occasions, movements, herd breakdown incidences and annual test frequency all satisfied the inclusion criteria with positive odds ratio in association with a false negative SICCT test outcome. Though its important to observe that “case” animals have experienced almost twice the number of the SICCT tests in the life span compared with animals in the “control” group (Table 5.5). All categorical and continuous variables were then passed forward for inclusion in the multivariable model development.

Table 5.4: Univariable logistic regression analyses for categorical risk factors associated with false negative outcome in SICCT test for selected case and control samples from the GB cattle herds between 2002 – 2012

Risk factors	Odds ratio	95% CI	N cases : controls
Animal age category			
1 (0–1 years old)	1	-	74 : 1673
2 (1–3 years old)	4.31	3.30 – 5.72	1107 : 7968
3 (> 3 years old)	5.33	4.08 – 7.09	841 : 4663
Animal sex			
Female	1	-	1396 : 10546
Male	1.31	1.18 – 1.46	626 : 3758
Animal type			
Beef	1	-	1228 : 9416
Dairy	1.24	1.12 – 1.37	794 : 4888
Herd type			
Beef	1	-	298 : 2464
Dairy	1.33	1.15 – 1.55	905 : 5549
Finishing/Store	1.36	1.15 – 1.62	403 : 2458
Other ^a	2.08	1.53 – 2.81	79 : 320
Suckler	0.77	0.65 – 0.92	337 : 3513
Previous breakdown incidents			
0 (No prev. breakdowns)	1	-	644 : 4054
1 (At least 1 breakdown incidence)	0.91	0.82 – 0.98	1378 : 10250
Time since previous test			
0 (No previous test)	1	-	293 : 2705
1 (> 120 days)	0.68	0.58 – 0.79	659 : 9446
2 (60–120 days)	5.34	4.57 – 6.26	904 : 1871
3 (0–60 days)	7.30	5.65 – 9.42	166 : 282
Time since recent move			
0 (No previous move)	1	-	1131 : 9168
1 (> 60 days)	1.43	1.29 – 1.58	852 : 4997
2 (0–60 days)	2.37	1.60 – 3.44	39 : 139
Total			2022 : 14304

^a Other herd type includes unclassified farms, or farms that hire animals and livestock dealers

Table 5.5: Univariable analyses and summary statistics for continuous variables associated with false negative outcome in SICCT test outcome

Risk factors	Odds ratio 95% CI	Cases	Controls
		Mean (SD) Range	Mean (SD) Range
Cumulative SICCT tests	1.11 1.10 – 1.13	5.70 (6.02) 0 – 35	3.35 (3.75) 0 – 32
Total number of movements	1.12 1.08 – 1.16	0.93 (1.34) 0 – 12	0.73 (1.26) 0 – 31
Total breakdown incidences	1.06 1.03 – 1.10	1.66 (1.74) 0 – 15	1.58 (1.52) 0 – 21
Annual test frequency	1.40 1.34 – 1.47	1.49 (1.15) 0 – 5	1.14 (0.97) 0 – 7

5.3.4 Multivariable analyses

The majority of factors selected from the univariable analyses also satisfied against the inclusion criteria under the multivariable model. However, factors associated with movement number and herd breakdown incidence were dropped in the variable selection process (i.e. $p\text{-value} > 0.05$) in the final multivariable model. Cumulative number of SICCT tests showed a strong correlation with the annual test frequency and was also excluded as a consequence. Annual test frequency was retained as it centralises the age effect by discounting the total number of tests in the animals' life history. Three categories under "herd type" failed to pass the inclusion criteria (i.e. with 95%CI containing zero) and as a result, was dropped from the final model. Though animal type is correlated with herd type, despite removing herd type variable, animal type was still non-significant and was removed as a result. The list of independent variables selected for inclusion in the final multivariable logistic regression model is presented in Table 5.6.

The time interval from previous SICCT test had a significant influence on the risk of an infected animal being mis-diagnosed under SICCT test (standard interpretation). From the final multivariable model results, the odds of false negative diagnosis in infected cattle that were re-tested within 60 days from previous test occasion were 5.37 times more likely than animals with no previous SICCT test. Cattle that were re-tested within 60-120 days were 3.88 times more likely to be missed, though this effect dramatically reduced for cattle tested more than 120 days from a previous test (Table 5.6). Furthermore, animals with previous movement history were also statistically associated with being false negatives. In particular, animals tested within 60 days from moving onto a new herd were 2.8 times more likely to be missed compared to home-bred animals with no movement history.

From animal-level factors, animals in older age categories were at significantly increased risk of been identified as false negative and males were 1.75 times more likely to be missed than female animals. In addition, for every unit increase in the average number of SICCT test per year, the odds of mis-diagnosis increased by a factor of 1.14 (95%CI: 1.06 – 1.23) for each infected animal. Animals in herds with at least one confirmed breakdown incidence in the last 4 years was negatively associated with being identified as a false negative under standard SICCT (OR: 0.76, 95%CI: 0.67 – 0.86). Interactions between animal age-sex and age-breed were also fitted under the multivariable logistic regression model, but has failed the inclusion criteria and also did not offer improvements in the model fit. The mean variance inflation factor between all variables in the final multivariable model is 1.137 (as a rule of thumb, VIF greater than 10 indicate potential problems of collinearity). The model diagnostic plots and residuals also does not raise any cause for concern under the current analysis.

Table 5.6: Multivariable logistic regression analyses with selected risk factors associated with false negative outcome from SICCT test (based on the inclusion criteria and the DIC scores)

Risk factors	Odds ratio	95% CI	N cases : controls
Animal age			
1 (0–1 years old)	1	-	74 : 1673
2 (1–3 years old)	5.70	4.29 – 7.67	1107 : 7968
3 (> 3 years old)	8.70	6.45 – 11.89	841 : 4663
Animal sex			
Female	1	-	1396 : 10546
Male	1.75	1.54 – 2.00	626 : 3758
Previous breakdown incidence			
0 (No prev. breakdowns)	1	-	644 : 4054
1 (At least 1 breakdown incidence)	0.76	0.67 – 0.86	1378 : 10250
Time since previous test			
0 (No previous test)	1	-	293 : 2705
1 (> 120 days)	0.50	0.41 – 0.60	659 : 9446
2 (60–120 days)	3.88	3.13 – 4.81	904 : 1871
3 (0–60 days)	5.37	3.98 – 7.23	166 : 282
Time since recent move			
0 (No previous move)	1	-	1131 : 9168
1 (> 60 days)	1.28	1.13 – 1.45	852 : 4997
2 (0–60 days)	2.80	1.83 – 4.23	39 : 139
Annual test frequency			
	1.14	1.06 – 1.23	2022 : 14304
Total			2022 : 14304

5.3.5 Sensitivity analyses

Amongst the subset of case and control animals with only positive *M. bovis* culturing at *post-mortem* (i.e. 100% confirmation of bTB infection), the majority of risk factors remained in the model. However, herd-level risk factors represented by previous breakdown incidences were eliminated for having a higher DIC statistic and 95%CI that contained zero. Odds ratios for the variables were positive and consistent with the main model, although there is an obvious increase in the estimates (from mean posterior) for several variables accompanied by wider credible intervals. This may be due to extra uncertainties with smaller sample size (40% less data), rather than an actual increase of magnitude in the effects (Table 5.7).

The sensitivity analysis where case and control selection were based on animals subject to SICCT test within 42 days (rather than 60 days) prior to slaughter revealed no substantial differences in the variable selection process and as a result, contained the same variables in the final multivariable model. Despite a 10% reduction in the sample size, the respective odds ratio estimates and 95% credible interval for all selected variables remained consistent and largely overlaps with results from the main model (Table 5.8).

Table 5.7: Results of multivariable logistic regression model for sensitivity analysis based on samples with positive *M. bovis* culture during *post-mortem* diagnostics

Risk factors	Odds ratio	95% CI	N cases : controls
Animal age			
1 (0–1 years old)	1	-	13 : 979
2 (1–3 years old)	18.51	13.52 – 24.98	765 : 5059
3 (> 3 years old)	23.37	18.91 – 34.24	531 : 2866
Animal sex			
Female	1	-	858 : 6539
Male	2.20	1.86 – 2.59	451 : 2365
Time since previous test			
0 (No previous test)	1	-	178 : 1901
1 (> 120 days)	0.55	0.43 – 0.69	481 : 6263
2 (60–120 days)	7.17	5.47 – 9.42	558 : 608
3 (0–60 days)	5.60	3.79 – 8.26	92 : 132
Time since recent move			
0 (No previous move)	1	-	672 : 5517
1 (> 60 days)	1.52	1.30 – 1.78	615 : 3284
2 (0–60 days)	2.97	1.71 – 4.99	22 : 103
Annual test frequency			
	1.29	1.18 – 1.41	1309 : 8904
Total			1309 : 8904

Table 5.8: Results of multivariable logistic regression model for sensitivity analysis based on 42 days as threshold value to select case and control samples

Risk factors	Odds ratio	95% CI	N cases : controls
Animal age			
1 (0–1 years old)	1	-	39 : 1591
2 (1–3 years old)	6.45	4.58 – 9.30	790 : 7605
3 (> 3 years old)	10.44	7.29 – 15.30	593 : 4380
Animal sex			
Female	1	-	971 : 9989
Male	1.85	1.59 – 2.15	451 : 3587
Previous breakdown incidence			
0 (No prev. breakdowns)	1	-	459 : 3847
1 (At least 1 breakdown incidence)	0.70	0.61 – 0.81	963 : 9729
Time since previous test			
0 (No previous test)	1	-	190 : 2562
1 (> 120 days)	0.48	0.39 – 0.60	454 : 8955
2 (60–120 days)	3.72	2.91 – 4.77	634 : 1797
3 (0–60 days)	6.68	4.81 – 9.29	144 : 262
Time since recent move			
0 (No previous move)	1	-	787 : 8675
1 (> 60 days)	1.32	1.14 – 1.52	610 : 4768
2 (0–60 days)	2.59	1.55 – 4.20	25 : 133
Annual test frequency			
	1.18	1.09 – 1.28	1422 : 13576
Total			1422 : 13576

5.3.6 Multivariable analysis for case and control samples with calving histories

In order to explore the effect of recent calving history on the outcome of the SICCT test, case and control samples were restricted to all female animals that had calved at least once previously. This naturally eliminates younger animals less than 2 years of age (female cattle usually experience first calving at minimum age of 2). Hence animal age categories were reclassified: “2-3”, “3-4” and “>4”. The final data consisted of only 753 case animal and 4,634 controls, of which 40% of animals had only one previous calf during its life span and approximately 1/3 of animals experienced 3 or more calving. Cattle recently calved within 30 days and also were subject to SICCT test within 60 days prior to slaughter consisted of only 14% of the data.

From the multivariable analysis, time since previous movement and annual test frequency were consequently removed in the variable selection process. Other factors such as animal age, previous breakdown incidence and time since previous SICCT test remained positive and were selected in the final model. The estimated odds ratios were smaller in general but the 95% credible intervals overlaps with estimates under the main multivariable model. However, adding the total number of previous calving and animals SICCT tested within 30 days of post-partum period (binary factor variable) did not improve the final model nor did they pass the inclusion criteria under the univariable and multivariable analysis (Table 5.9).

Overall, the multivariable analyses revealed stress-related events such as previous SICCT test and recent movement as well as several animal-level factors such as age and sex were associated with an increased odds of being a false negative under the standard SICCT test. Herd-level factors were largely non-significant

Table 5.9: Results of multivariable logistic regression model for subset of case and control sample with at least one previous calving history

Risk factors	Odds ratio	95% CI	N cases : controls
Animal age			
1 (2–3 years old)	1	-	33 : 633
2 (3–4 years old)	1.74	1.12 – 2.76	94 : 1066
3 (> 4 years old)	3.96	2.63 – 6.11	626 : 2935
Previous breakdown incidence			
0 (No prev. breakdowns)	1	-	185 : 1221
1 (At least 1 breakdown incidence)	0.73	0.57 – 0.93	568 : 3413
Time since previous test			
0 (No previous test)	1	-	36 : 226
1 (> 120 days)	0.22	0.15 – 0.35	248 : 3705
2 (60–120 days)	2.06	1.32 – 3.28	403 : 619
3 (0–60 days)	3.04	1.73 – 5.37	66 : 84
Time since previous calving			
0 (Calving > 30 days)	1	-	726 : 4371
1 (0–30 days)	1.04	0.47 – 1.41	27 : 263
Total number of calves			
	1.13	0.99 – 1.20	753 : 4634
Total			753 : 4634

but the presence of previous herd breakdown history reduced the odds of false-diagnostic of infection. Factors associated with calving activities appears to have little or no effect on the false negative outcome from the SICCT within the study, though data were limited to explore this effect in detail. Interactions between age and sex were explored, but showed no additional improvement in the model fit.

5.4 Discussion

The SICCT test has been used very effectively as a herd test (OIE, 2009), but has serious limitations in identifying individual infected animals in a herd, and it is unable to differentiate between infected animals showing varying degrees of infection and pathology (Schiller *et al.*, 2010a). Laboratory-based experiments with infection models of bTB have confirmed the failure of the SICCT test to detect infected animals, including some with well-developed pathological lesions (Neill *et al.*, 1988). Field studies have shown that SICCT test-negative animals, in contact with SICCT reactors from multi-reactor breakdown herds, were infected and missed by the disclosing SICCT test (Bovine TB Advisory Group, 2009). The analysis described in this chapter was designed to examine potential risk factors that may cause infected animals to be missed by the SICCT test. It assumes that a positive *post-mortem* is confirmation of infection and compared cases (SICCT test negative within 60 days prior to slaughter) with controls (SICCT test positives) and explores, specifically, stress-related risk factors and their effect on the test outcome.

A random effect logistic regression model was fitted under the Bayesian framework using INLA for the benefit of computational efficiency. The two main stress factors investigated in this study were recent movements and historical SICCT testing. The sample consisted of only 178 animals ($\sim 1\%$ of the sample

size) that were classified as recently moved within 60 days prior to the reference SICCT test, the association between the odds of false negatives and recent movement suggests that animals moved within 60 days were 2.8 times more likely to be false negatives in comparison to home-bred animals that never moved. Although not as strong an effect, animals moved more than 60 days prior to a SICCT test were 1.28 times more likely to test false negative. Movements of infected animals have long been considered a critical factor in the spread of bTB (Gilbert *et al.*, 2005), as reflected in the introduction of legislation that requires all cattle imported from high incidence regions to be tested for bTB within 120 days of arrival into a low incidence area (Gates and Volkova, 2012). However the transportation between farms, particularly over long-distance, can be a very stressful period for the animal and this chronic exposure to stress can also lead to suppressed cell-mediated immunity (Verbrugghe *et al.*, 2012). As a consequence, this can affect the diagnostic outcome of SICCT which relies on cell-mediated immune response to injected tuberculosis antigens. Other animal challenge studies have shown that change of environment and movements could suppress antibody production following immunisation of stressed mice (Griffin, 1989)

Given evidence on biological grounds, that repetitive SICCT testing within short time intervals may induce desensitisation in animal's immune response (Radunz and Lepper, 1985; Coad *et al.*, 2010; Thom *et al.*, 2004; de la Rua-Domenech *et al.*, 2006), legislation for bTB testing require a minimum separation of 60 days between two consecutive SICCT tests (Defra, 2008a). However, despite this legislative requirement, the case study has identified 448 cattle (3% of sample) that have experienced two SICCT tests less than 60 days apart. In addition, 2,775 cattle (17% of sample) were subject to a second SICCT test between 60 and 120 days. Mitchell *et al.* (2006) demonstrated that many cattle (in low incidence areas) were never tested during their lifetimes because of turnover and movement

between herds. Indeed, this is reflected by the low numbers of young animals with identified SICCT test history (only 3.6% of samples with at least two SICCT tests were less than 1 year old) while 67% of young animals less than 1 year of age had no previous testing record prior to the reference SICCT test. Using “no previous SICCT test” as a baseline for comparison, time interval since previous test and the annual test frequency were statistically associated with false negative test outcome. Specifically, SICCT tests conducted between 0–60 and 60–120 days prior to the reference test had an odds ratio of 5.37 (95%CI: 3.98 – 7.23) and 3.88 (95%CI: 3.13 – 4.81) respectively compared to animals not previously tested, and this effect diminished in animals tested more than 120 days with non-informative odds ratio of 0.5 (95%CI: 0.41 – 0.6). Furthermore, an increase in the average number of SICCT test per year results in an increase of 1.14 (95%CI: 1.06 – 1.23) odds of being miss diagnosed. Although this effect is likely to be correlated with age, as an animal gets older, its more likely to have experienced a greater number of SICCT tests. Similar findings have been reported in other experimental studies where a transient failure to a second SICCT test was observed in animals inoculated with *M. bovis* and Thom *et al.* (2006) concluded that the time at which the SICCT test is administered may be of significance. It is worth noting that the study only considers herd testing outlined in Table 5.1. It is likely that animals will be eligible for other types of individual tests such as check test, tracing test, inconclusive test and pre- and post-movement test which were designed to target specific groups of animals. So therefore the SICCT test occasions used in the current analyses is an under representation of the true testing history in the animal’s life span, hence the potential for even stronger effect under repeated testing.

Animal age, breed and sex were included in the logistic regression models to account for biases introduced by the criteria for selecting case and control animals to being tested by the SICCT test. Older female cattle are at increased risk of

being reactors as well as being tested (Green *et al.*, 2012), and common dairy breeds have smaller reactions in the SICCT test compared to beef breeds (Amos *et al.*, 2013). In addition, analysis by Brooks-Pollock *et al.* (2013) demonstrated that bTB infection risk has a strong age dependency and that the probability of detecting infection increases with age. Other epidemiological studies have shown that animal age is a classical confounding factor for being identified as a reactor on routine herd test (RHT) (Gates *et al.*, 2013; Munroe *et al.*, 2000). Although age was considered a known risk factor for bTB in several studies, amongst infected animals, results from the current analysis shows that animal age is also associated for been false negative. The odds ratio for false negative SICCT outcome increases with age which indicates a possible decline in the test sensitivity as animals get older, particularly for animals more than 3 years of age. This is consistent with findings that sensitisation to tuberculin was described as a possible risk for failing a test in particular age groups (2-5 year old cows), which may have influenced the ability for SICCT to detect infection in older animals (Cagiola *et al.*, 2004; Coad *et al.*, 2010). A similar risk factor analysis for failure in the *ante-mortem* diagnostic of bTB in Northern Ireland also confirmed that increasing age was significantly associated with increased risk of disclosing false negative cattle (Lahuerta-Marin *et al.*, 2016). While other field of epidemiological studies also found age as a risk factor for infected animals failing a test (Alvarez *et al.*, 2014; Shittu *et al.*, 2013). Age is likely to be a proxy measurement of the combined period of exposure to *M. bovis* and bTB antigen, as well as the number of tests experienced (Green *et al.*, 2012). This characteristic can increase the risk for cattle reacting to the SICCT in relation to non-infected animals, however, amongst confirmed infections, can also suppress the immune response to the SICCT with accumulating increase in risk with age (Griffin *et al.*, 1996). Comparisons can also be drawn with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in cattle. Response to MAP has been shown to vary with age with young calves at increased risk of developing high bacterial

loads (Nielsen and Ersbøll, 2006; Mitchell and Medley, 2012) and as a result, were more likely to respond to the diagnostic test. Though in contrast with human TB, the SICCT test has a lower sensitivity for disease in young children in comparison to other age groups (Moyo *et al.*, 2011).

Given similar age structure between male and female groups, the results revealed that males were more likely to be false negatives than female. Although no direct evidence that male animals in general were more likely to be missed under standard SICCT testing, studies have shown that female inconclusive reactors were more likely to react positive at the next test than male (Clegg *et al.*, 2011c), and female animals had a higher risk of a bTB-positive test compared to male animals (Wolfe *et al.*, 2009). A similar case-control type study evaluating factors for false negative diagnosis of bTB in Northern Ireland also found that males had marginal higher odds of been missed during SICCT than females (though significant only in univariable models) (Lahuerta-Marin *et al.*, 2016). Theoretically, one plausible explanation could be that male cattle tend to have thicker skin, and are less easy to handle, and are therefore harder to observe a comparative difference in SICCT test measurements during testing which can result in miss identification of infection (Wright *et al.*, 2013). However, despite being a different species, an epidemiological study on bTB transmission in wild badgers found novel evidence that male badgers have higher risk of bTB infection and more rapid disease progression compared with female, coupled with increases in disease-induced mortality (Graham *et al.*, 2013). While it is difficult to replicate findings from different study populations, whether or not the apparent differences in detectability of bTB between genders relates to physiological differences remains unclear.

Despite studies suggesting animal breed can also affect the relative degree of immune response (Amos *et al.*, 2013; Alvarez *et al.*, 2014; Lahuerta-Marin

et al., 2016), there was no evidence for a statistical association between false negative test outcomes and animal breed after other factors are taken into account. However, the analysis was based on generalised breed type (i.e. beef and dairy, with mixed breed been classified as beef) rather than using the generic breed of the cattle as it is the case in the study by Amos *et al.* (2013).

As well as animal-level characteristics, several herd-level risk factors were also examined. Cattle were less likely to be false negative (i.e. more likely to react) under the standard SICCT test when they were tested on farms with previous confirmed breakdown incidence in the past four years. This result concurs with findings from other case-control studies in endemic regions, where cattle that had been present at a previous bTB herd test with disclosed reactors were shown to be significantly more likely to react positively on subsequent herd tests (Wolfe *et al.*, 2009; Ramírez-Villaescusa *et al.*, 2009). Given that previous history of bTB increased the risk of herd breakdowns (Skuce *et al.*, 2012; Byrne *et al.*, 2014), it is also possible that the regulatory officials are more likely to classify an animal as a positive reactor if there is reason to suspect potential exposure to bTB infected cattle. Additional consideration might be that herds with no previous breakdown histories have different management practices that increase the risk of hidden/latent infection. Different herd types are likely to have different management practices, and their effects have been well documented (Reilly and Courtenay, 2007; Alvarez *et al.*, 2012; Ramírez-Villaescusa *et al.*, 2010). Results from analysis in Northern Ireland by Lahuerta-Marin *et al.* (2016) indicate a potential higher risk of animals being missed by SICCT test in dairy herds in comparison to beef herds, although the contrary conclusion was made in a Spanish study where the test performance was better in dairy relative to bullfighting and beef herds (Alvarez *et al.*, 2014). It has been described that dairy breeds may have different levels of genetic resistance to *M. bovis* relative to beef herds (Allen *et al.*, 2010; Richardson *et al.*, 2014), however, there was no evidence that herd

production types (i.e. beef, dairy, finishing/store and suckler) affect the SICCT test outcome in the current study.

The subset analysis exploring the association with recent calving activities revealed no significant relationship. This may be due to the absence of visible lesion or positive culture in animals recently calved and therefore large number of test positive (reactors) were neglected based on the selection criteria in the current analysis. Other possible explanations could be that, after all, recent calving and parturition does not have any significant effect on the immune response to the SICCT test. A recent Irish study showed that weaning induced an acute stress response in calves and actually enhanced the immune response (O'Loughlin *et al.*, 2011). However, results from analysis in the previous chapter and findings from early experimental studies by Buddle *et al.* (1994); Kerr *et al.* (1946), as well as policy recommendations in countries with successful bTB control programme (Vetent, 2015), suggests that the effect of recent calving on the risk of false negatives from the SICCT test cannot be ruled out.

The analysis assumed a minimum of 60 days for development of visible lesions and positive culture from initial infection. Findings from animal challenge studies indicate that gross visible lesions consistent with tuberculosis can be observed in the lymph nodes and lungs as early as 42 days after inoculation (Palmer *et al.*, 2007). In addition, disclosure of typical bTB lesions does not, in itself, prove infection because such lesions can on rare occasions be caused by other mycobacteria (Defra, 2008a). To address these potential limitations, a more conservative threshold of 42 days and culture of *M. bovis* in the laboratory (i.e. definitive proof of infection) were used for the case-control selection criteria in a sensitivity analysis. The results from the stress-related events did not show huge differentiation as expected. In the study by Coad *et al.* (2010), it was also shown that cattle in the study with field reactors could have been infected longer than

those experimentally infected which tend to be monitored for shorter time periods. Furthermore, immune responses may especially be higher early after infection, as demonstrated by study in experimentally infected cattle (Schiller *et al.*, 2009). Another criticism of experimental studies was that responses were relatively strong (due to high doses); thereby, limiting the possibility for demonstration of subtle effects that may have been achieved with lower responses commonly observed in naturally infected field cases.

The current case-control analyses does not incorporate elements of spatial and temporal dependencies. Though farm location ID was used as an random effect in the logistic regression analyses, information on the geographical locations (i.e. coordinates) of the farms were often limited. With more detailed farm-level data available in the future, further analyses can be tailored to include spatial data that will provide an extra dimension when evaluating the diagnostics of bTB.

In conclusion, this analysis is consistent with the hypothesis that physiological stress-related risk factors may have an effect on the sensitivity of the standard SICCT test. Unidentified infection in cattle is likely to contribute to local persistence as well as having the potential to initiate new breakdowns via within and between herd animal movements and contacts. The tuberculin test will fail to detect some diseased animals that are potential transmitters of disease to other cattle and possibly to local wildlife. It is therefore important to understand reasons for miss diagnosis.

To my knowledge, no published study to date has used empirical field data (i.e. the CTS database and bTB testing data) to explicitly evaluate the potential effect of physiological-stress factors on the sensitivity of the SICCT test. Although it is difficult to obtain direct biological evidence of the immune-suppression effect due to lack of empirical data, they highlight several important factors for future epidemiological research. By considering animal-level characteristics (such as

age, sex and the physiological status), it is possible to provide veterinarians (i.e. bTB test conductor) with better guidance on interpreting the test outcome. In particular, under future surveillance systems, results from this study could be used to adjust the timings of testing relative to movements and previous test occasions in order to minimise the risks of false negative test or increasing the threshold for reactor definition (e.g. apply severe interpretation standards) in animals under these categories to complement the poor test sensitivity.

Chapter 6

General discussion

6.1 Introduction

The main objective of this thesis was to provide empirical examples which demonstrates why bovine tuberculosis (bTB) remains a complex and challenging livestock disease to control in the cattle production systems, and how insights gained from the analysis of the national cattle movement records and surveillance testing can be used to develop more effective disease control programmes in the future. Throughout the data chapters, one of the major recurring themes was that ‘missed infections’ (resulting from either missed test or mis-diagnosis) can have profound effects on the disease transmission dynamics and the performance of surveillance activities at the industry level. In particular, there were examples of undetected infections that may have contributed to the ‘silent’ spread of the disease both within and between cattle herds. From individual animal-levels, there was evidence that many factors including animal characteristics and physiological stresses which can potentially contributes to a lack of response from the standard

diagnostic test, and factors such as timings of testing may need to be adjusted to avoid possible mis-diagnostics. However, the current approaches to modelling disease transmission and evaluation of bTB diagnostic test still require further refinement before the outputs can be used to inform national disease control policy. The following sections discuss the implications of the main thesis findings for future investigation of bTB surveillance and the implementations of control programmes.

6.2 Control implications

From an epidemiological perspective, operating a completely closed herd is the most effective means of preventing bTB transmission within and between cattle herds. From a practical perspective however, this is impossible to achieve. Hence, almost every published literature and resources recommends purchasing cattle either from certified TB-free herds or for animals to be quarantined and tested after purchase to reduce further transmission risk (Defra, 2010; Gates and Volkova, 2012). In addition to the challenges of limiting disease transmission, the primary diagnostic test for bTB is far from perfect and repeated testing within short period of time (i.e. 60 days) is strictly prohibited to reduce missed infection due to desensitisation (Thom *et al.*, 2004; de la Rua-Domenech *et al.*, 2006; Schiller *et al.*, 2010a). Findings from this thesis have provided additional evidence that these measures are frequently violated in practice and that a blanket approach to diagnostic testing is not necessarily the most efficient way to disclose infections. Results from the empirical data analysis as well as various sources of literature suggest that a range of factors can potentially effect the sensitivity of the standard bTB diagnostic test and these needs to considered when interpreting the test outcome for each individual animals in order to minimise the number of missed infections. Aside from these challenges, the dynamics of bTB transmission

and control is further complicated by the wildlife aspect of infection. This means that in high-risk areas (with an established wildlife reservoir of infection), even for completely closed herds, there is a constant threat of disease introduction from wildlife sources and the potential for disease to spread further afield due the wildlife movement (which are mostly untracked). Thus, there are still significant gaps in our understanding of bTB transmission and diagnostics. In particular, there is a strong need for further research into the cattle-wildlife interactions as well as identifying the potential confounders of the current bTB test in order to aid the development of improved bTB diagnostics.

6.2.1 Within-herd cattle-to-cattle transmission

In the absence of an established wildlife reservoir of infection, the movements of undetected and infected cattle from other endemic areas are considered to be the primary method of bTB introduction (Gilbert *et al.*, 2005). Gates *et al.* (2013) demonstrated evidence that purchasing cattle from regions with endemic bTB was a significant risk factor for herd breakdowns in Scotland despite the fact that these imported animals were all subject to post-movement testing. Empirical analyses in Chapter 2 using field data provided additional evidence that within-herd cattle-to-cattle transmission occurs after disease introduction. Although it is often difficult to determine the true source of disease dissemination, the detection of infected home-bred animals with no movement history demonstrates that the progression of disease does occur once the herd became infected. Furthermore, it was shown that the magnitude of the within-herd incidence is associated with the average herd size and the duration of disease exposure period, which is closely related to the speed of disease detection. Epidemiological models of bTB developed by Conlan *et al.* (2012) also emphasised the ‘hidden burden’ of infection, and suggested that bTB testing may be missing many animals

harbouring the disease and that large herds (with higher density) may suffer a higher incidence of disease as well as faster transmission. However, in reality, herd size does not necessarily relate to stocking density directly (McCallum *et al.*, 2001). Nevertheless, these findings suggest that the within-herd cattle-to-cattle transmission is likely to be non-linearly density dependent, and this can have important implications for the formulation of herd-level policy of bTB (i.e. additional controls may need to be targeted towards larger herds).

Although the conditions which can impact on the incidence rate were not expected to be constant across all the farms, nor does herd size and length of exposure the only variables of concern. However, there is indication that the within-herd incidence rate is considerably higher for some herds compared with others, and one explanation for this higher rate may be the existence of ‘super-shedders’. In an animal population, a ‘super-shedder’ state has been described encompassing those animals where bacterial shedding is detected by culture persistently or by several routes, in contrast to the standard, intermittent shedders (Santos *et al.*, 2015). In reality, it is not known which animals transmit the most, although current epidemiological evidence and modeling studies are lending support to the concept of the ‘super-shedder’ (or super-spreader/super-excretor) animals, but there is a lack of direct evidence to support this supposition in the UK cattle herds (Renwick *et al.*, 2007; Skuce *et al.*, 2011; O’Hare *et al.*, 2014; Santos *et al.*, 2015). However, it was suggested that factors which affect animal’s social behaviour might facilitate cattle-cattle transmission (White *et al.*, 2008; Drewe *et al.*, 2011). Cattle contact patterns are highly variable (White *et al.*, 2008) and can be influenced by their relative position in the herd social hierarchy. Cattle that are higher in the herd social hierarchy show greater inquisitiveness and have a higher risk of acquiring infection from cattle introduced to the herd (Sauter and Morris, 1995), as well as potentially from direct contact with infectious wildlife (Böhm *et al.*, 2009). In addition, some cattle are highly connected within the herd

contact network and have the potential to act as hubs in the spread of disease within these complex contact networks (Drewe, 2010). This concurs with data from New Zealand, where bTB reactors tended to be from the top half of the herd hierarchy (Sauter and Morris, 1995). Hence, targeting prevention or control measures to high-contact individuals (or herds) may be an effective way to further enhance disease management of bTB.

The analysis was designed to give some estimate of the rate of spread in an infected cattle herd, as it is difficult to predict the within-herd incidence rate given the ambiguity in determining the disease introduction point and variations between farm conditions (e.g. management practices and factors affecting farmer behaviours). From a practical perspective, it is unclear how much the within-herd transmission mechanism contributes to the overall spread of bTB on industry level, nevertheless, the distribution of the estimates provide a good indication of the rate of spread within cattle herds in Low Risk Areas (e.g. Scotland, North, East and South East of England) and can be used to parameterise dynamic models of bTB transmission on a larger scale. The advantage of the current method of estimation to policy makers is that for any given herd size the expected number of bTB cases can be calculated with any particular length of disease exposure period, thus providing an indication for the severity of the disease situation and more targeted control strategy.

6.2.2 A stochastic simulation framework of bTB transmission

The method of using computer simulations to make inference on disease transmission and rehearse surveillance strategies with a view to inform government policy is by no means new in the study of bTB spread (Barlow *et al.*, 1997;

Conlan *et al.*, 2012; Brooks-Pollock *et al.*, 2014; O'Hare *et al.*, 2014). However, extant simulation models either focuses on parameter estimation or fails to account for dynamic system of disease transmission via animal movement. The bTB simulation modelling framework presented in Chapter 3 incorporates stochastic transmission of infection within-farm (SEIT-model) and between-farms (via animal movement), maintenance of infection in the environment (external and wildlife infections) and a system of routine surveillance tests (consists of routine bTB skin test and regular monthly slaughterhouse test). Most importantly, it also allows the flexibility to alter the timings of each herd tests as well the types of farm-contact structure to evaluate their potential impact on the disease spread across a network of farms. With a constant rate of disease transmission (both within and between herds), several different routine testing strategies were evaluated. Of all the approaches tested, the most effective in reducing the level of infection was to increase the routine herd test frequency across all farms in the network. From an epidemiological perspective, one of the most effective means of reducing bTB incidence amongst cattle herds is to test as many herds and as frequently as possible (Bourne, 2007). This is, of course, impossible from a practical perspective due to limitations in available resources. As a result, the level of surveillance activities often depends on the risk of infection in the local areas as well as the assessed epidemiological risk of infection for individual herds (Green and Cornell, 2005).

While the frequent testing of cattle herds and the removal of reactors to limit the cattle-to-cattle transmission remains the cornerstone of any bTB control programme, in reality, a high prevalence of bTB still persisted in large parts of the south-west of the country despite enhanced herd control measures (Defra, 2014b). This was also evident in the simulation models, where the main driver for high disease prevalence for some herds with annual testing frequency is the external force of infection. The existence of a wildlife reservoir of infection has

been identified as a significant external factor that contributes to the frequent re-introduction of bTB in many cattle herds in high risk areas (Reilly and Courtenay, 2007; Donnelly and Hone, 2010). Hence, an effective bTB control programme should address the reservoir of infection in wildlife as well as adapt an effective application of disease control measures in cattle to optimise the surveillance effort and improve efficiency in disease detection (Palmer, 2013).

Other results from the simulation models developed in Chapter 3 demonstrated the impact on disease spread for several hypothetical scenarios where the epidemiological situation of bTB may change (i.e. bTB becomes more transmissible between cattle-to-cattle, an increase in external force of infection such as wildlife transmission and the development of more sensitive diagnostic test protocols). This highlights some fundamental challenges in controlling the disease and that the key to addressing the ongoing spread of bTB lies with reducing the rate of transmission within and between herds as well as the level of external force of infection (with infectious wildlife being the primary cause of concern) (Conlan *et al.*, 2012). The central question remains as to whether this requires close management of farmer practice, or intense control of the reservoir of infection in wildlife populations, or simply improved surveillance and diagnostic testing to reduce the number of undisclosed infections.

Although the mechanical models developed in this thesis are not intended for parameter estimation, their basic approach for simulating bTB spread within a farm contact network can easily be modified to address many important epidemiological questions. For instance, there has been growing interest in introducing a risk-based method to routine herd testing to optimise resource allocation (Bessell *et al.*, 2012a). This has been adapted in Scotland in recent years. The simulation model framework can be used to explore the potential impact on the patterns of bTB spread by adapting to a risk-based routine test

strategy as well as providing ways to assess the optimisation of surveillance effort for disease detection, thus helps to inform relevant policy decisions.

For the model to be of use in guiding future policy decisions, there are several additional layers of complexity that must be considered. First, although between farm contacts were accounted for via stochastic monthly movement of animals, real animal movement data (i.e. the CTS database) needs to be implemented to represent a realistic picture of the UK farm contact networks (rather than theoretical contact structures). These data could be used to assign farms with a degree distribution and to generate rules for contact formation that would feed directly into the network generation algorithm. Studies regarding the use of network analysis in modelling the spatio-temporal spread of livestock diseases has already been conducted on cattle movement networks in the UK (Kiss *et al.*, 2006a; Nohuddin *et al.*, 2010b; Firestone *et al.*, 2011), as well as other types of social network studies using the CTS data (Nohuddin *et al.*, 2010a; Firestone *et al.*, 2011). Secondly, a spatial element can be incorporated that distinguishes farms by their unique geographical locations and thus provide a graphical representation of the local/global spread of bTB. Farm coordinates data are limited under the current CTS database, but this should be addressed into future models with the improved data collection outlined under the new CTS development. Thirdly, by recycling the 4-year routine herd test data in the disease simulation model, it was assumed that the frequency and date of testing remained fixed over time. However, both can change quite substantially from year to year, and with the omission of other surveillance testing (such as pre- and post-movement tests as well as the various forms of check tests over the year), the disease are likely to be detected more frequently. Finally, any heterogeneity in heritability and susceptibility of the disease from individual animals as well as herds can have substantial impact on the transmission, especially with the existence of ‘super-spreaders’ (Conlan *et al.*, 2012; O’Hare

et al., 2014). Thus, variability in transmission rate within- and between-farms needs to be incorporated before control strategies can be optimised.

6.2.3 The association between physiological stress factors and unresponsive outcome from *ante-mortem* diagnostic tests for bTB

The lack of affordable and accurate diagnostic tests remains one of the major barriers to eradicating bTB and an important contributor to missed infections (Schiller *et al.*, 2011). While there have been many published experimental studies and epidemiological analyses investigating factors that can potentially depress the immune response to bTB diagnostic test, the analyses presented in Chapters 4 and 5 are the first to my knowledge that attempt to explore the effect of physiological stress-related events on the test outcome using empirical data for cattle herds in the UK.

It is generally accepted that a state of anergy may develop in cattle with advanced or generalised TB and (temporarily) in animals subjected to stress (Pollock and Neill, 2002; de la Rua-Domenech *et al.*, 2006). The evidence reviewed by Monaghan *et al.* (1994) suggested that infected cattle that have calved within the preceding four to six weeks sometimes fail to react to the tuberculin test. The analyses in Chapter 4 also explored the relationship between recent calving and the outcome (positive or negative under standard interpretation) to the SICCT and gamma-interferon test. The case-control studies demonstrated that recent calving within 60 days of a test is a significant risk factor for negative outcome in both SICCT and gamma-interferon diagnostic. In addition, the odds ratio progressively declined (towards non-significance) when the testing occasion moved further away from the parturition date. This tendency of a suppressive effect

on the immune response during periods of pregnancy and parturition was also described in early experimental studies (Kerr *et al.*, 1946; Collins *et al.*, 1986; Buddle *et al.*, 1994). There are also other examples where disease control measures have been imposed on recently calved animals when conducting bTB diagnostic tests. For instance, New Zealand, with a hugely successful bTB control policy, introduced restrictions to avoid testing animals 3 weeks either side of calving to avoid potential miss diagnosis. Government advice cites hormones in late pregnancy/early lactation as limiting factors that can potentially affect the bTB diagnostic test (Vetent, 2015).

Chapter 5 provided evidence that conducting SICCT tests on animals with recent movement (within 60 days) and recent SICCT test (within 60 and 120 days) histories were significant risk factors for false negative diagnostic outcome. Despite the fact that regulation require a minimum interval of 60 days between two consecutive SICCT tests (Defra, 2010), a large number of sequential tests occurred within the 60 days period. A Defra-funded research project (Thom *et al.*, 2006) investigated the effect of repeated SICCT test on the immune responses following experimental infection of *M. bovis*. Although it was emphasised that the experiment was not designed to determine whether one SICCT tests compromised a second one, the observations clearly demonstrated a marked reduction in the intensity of the SICCT test and in the number of animals that would be recognised as reactors was evident when animals were tested 15 weeks post-infection compared to their responses 8 weeks earlier, which could have consequences for diagnosis of bTB. While a study by Coad *et al.* (2010) also concluded that repeated SICCT testing within 60 days leads to desensitisation in naturally infected tuberculous cattle, results from the risk factor analyses in Chapter 5 provided additional indication that the suppression effect of repeated testing may even last beyond the recommended 60 days threshold (repeated SICCT test within 120 days was also significantly associated with false negative

outcome). However, there is need for further research to demonstrate direct evidence of the impact of stress on the performance of the diagnostic test for bTB, and to develop a reliable way of quantifying these effects, which could then be applied to support decision making criteria when determining the disease status of individual animal under field situations (e.g. apply the severe interpretation of SICCT for animals experiencing physiological stress, or be more intelligent in the timings of the test to avoid potential missing infections).

Overall, one of the most important contributions this thesis has made is establishing a direct relationship between physiological stress-related events and the potential risk of missing infection in the SICCT test. In the veterinary context Fraser *et al.* (1975) have defined stress as “an abnormal or extreme adjustment in the physiology of an animal to cope with adverse effects of its environment and management”. This term is used to identify the extreme response to adverse stimuli which can cause a damaging pathophysiological reaction in the host, producing associative changes in behaviour, physiology and disease susceptibility (Griffin, 1989). In addition, stress has long been associated to have the potential to alter immune responses in animals, studies have found that chronic stress tends to suppress the immune system and increases the susceptibility to diseases such as bTB (Wolfe *et al.*, 2009; Verbrugghe *et al.*, 2012). However, such studies are scarce and sometimes contradictory results have been reported. For instance, it was shown that repeated exposure to acute stress can result in an adaptation response which can cause enhancement of the immune response (Dhabhar and McEwen, 1997; O’Loughlin *et al.*, 2011). Kudahl *et al.* (2007) acknowledged that stressors, such as calving, handling or movements can accelerate the development of mycobacterial infections, thus, in theory, making disease detection more easier. Generally speaking, to understand the interactions between different types of stress, the host immune system and *Mycobacterium bovis*, it is of importance that animal models are created and field studies are conducted to investigate the effects

of stress and stress hormones (individually or simultaneously) on bTB infections in different hosts (including wildlife - capture of wild animals may evoke adaptive stress in the host).

From a broader perspective, understanding the factors that lead to the non-disclosure of infected animals is essential to optimise large-scale bTB disease eradication programmes. According to current estimate of the sensitivity of the SICCT test (51% from meta analyses by (Downs *et al.*, 2011)), approximately 50% of bTB infected cases were not able to be detected. The high number of potential false negative test results cannot be solely attributed to the physiological stressors investigated in the study, it is likely that other factors such as co-infections (Alvarez *et al.*, 2009), stages of infection (Thom *et al.*, 2006), animal housing (Skuce *et al.*, 2011), husbandry practices (Reilly and Courtenay, 2007), nutritional status (Thomas *et al.*, 2010) and testing techniques (Strain *et al.*, 2011) all contribute to the missed infections. These findings highlight that many factors are likely to influence the diagnostic outcome of bTB, and these must be considered in future surveillance to support policy decisions and minimise potential missing infections. While data from the CTS and Sam's IT system can be used to evaluate some stress-related events (i.e. parturition, movements and previous tests), information on other animal-level and herd-level characteristics were limited (for instance, such as the intended production purpose of individual animals and the production type of the farm). It would be useful to introduce this information into the database in the future, which would provide highly valuable information to support further research into the drivers of missing infections.

6.3 Study limitations

All analyses in this thesis were based on data from the CTS database and the Sam's IT system, these were secondary data provided by the Department for Environment, Food and Rural Affairs (Defra). While this approach enables researchers to answer high-impact questions without having to invest significant amount of time and resources in primary data collection (Smith *et al.*, 2011), there are limitations in using the data for purposes beyond its original scope. During the analyses, there were particular challenges associated with the lack of *post-mortem* diagnostic results in the Sam's IT system and the inconsistencies in how individual animals and farm businesses were identified between the different databases. More generally, the scarcity of data on negative tests and direct result of test measurements (e.g. SICCT test and gamma-interferon measurement) made it difficult to quantify the potential effect of missing infection. In addition, there was also a general lack of data on factors influencing farmer behaviour and herd management practices. For example, there were particular challenges associated with the lack of comprehensive herd production information in the CTS database and the Sam's IT system, and data were also limited on other herd characteristics such as housing types, common grazing area and GIS (geographic information system) land parcel data, which can all have important implications on disease control. This all indicate potential opportunities for improving the quality and scope of data collected in future epidemiological investigations.

6.3.1 Data limitations

Neither the CTS database nor the Sam's IT system was originally designed to support epidemiological research and there were several limitations that could

have influenced the study findings. Although the CTS database describes detailed demographic information on individual cattle and herds, it was originally designed for use in slaughter contact tracing investigations (Bourne, 2007). Under the CTS data recording, farmers are not required to report abortions, births of stillborn calves and births of calves that die shortly (within few hours) after parturition (Gates and Woolhouse, 2014). These events means that the sample size for the case-control studies conducted in Chapter 4 and 5 would be underrepresented, this can potentially influence the outcome from the regression analyses where important risk factors may be underestimated or significant factors may become non-significant. In many other cases, movements between common grazing pastures and from ‘linked’ premises were also not recorded (Orton *et al.*, 2012), which may have led to underestimation of import movements or exposure to imported cattle. Consequently, this also made it difficult to confirm that all cattle present on the farm holding were tested as part of a herd testing procedure as was assumed when inferring negative test outcomes in the risk factor analyses. Furthermore, farmers are also not required to declare the production type of their farm (i.e. beef suckler, dairy, heifer rearer or fattening), nor does the database holds information on the intended production purpose of individual animals (i.e. breeding diary, bull, or fattening beef) or the reasons for each of the cattle movement (such as breeding replacement, involuntary cull, or movement to seasonal grazing pasture). These factors can influence farmer behaviour and give indications on the herd management practices, which have potential impact on the risk of disease transmission and control.

Secondly, the variation in standards of operation and inspection procedures at slaughterhouses (Frankena *et al.*, 2007; Olea-Popelka *et al.*, 2008) combined with the lack of bacterial culture results made it difficult to accurately determine the true disease status of individual animals present in breakdown herds (Shittu *et al.*, 2013; Pascual-Linaza *et al.*, 2017). In practice, for every breakdown

disclosed by SICCT test, at least one reactor animal is sampled for bacteriological culture as confirmation of infection. However, in breakdowns with more than one reactor, the current sampling regimen (State-Veterinary-Service, 2005) restricts the number of animals with or without visible lesions that may be sampled for *M. bovis* culturing and molecular typing (Gopal *et al.*, 2006). As a result, the *post-mortem* diagnostic results in breakdown herds with multiple reactor animals may be incomplete, this has led to a general low rate of confirmation of infection. Subsequently, the potential large number of false positive classifications or animals truly infected with bTB but failed to be included in the analyses as a result of unconfirmed cases can all undermine the study findings. Careful monitoring of the standard inspection procedure and thoroughness of the meat examination, combined with appropriate training and experience of the meat inspector are ways to increase the overall confirmation rate at slaughterhouses (Shittu *et al.*, 2013). This would provide more valuable information to support further research into the drivers of missing infection.

During data manipulation and analyses, there were particular difficulties encountered in linking farm and animal data from the CTS databases with data from the Sam's IT system. For instance, there were many cases where cattle in the CTS database had no routine herd test records and many holdings with herd-test results in the Sam's IT system had no cattle according to the CTS Livestock Locations table. This is primarily because a single farm business may house cattle on multiple uniquely identified locations, but the surveillance and survey results are stored under the main farm CPH-identifier regardless of whether cattle are housed on that location (Gates and Volkova, 2012). Although BCMS maintains a list of 'linked' premises, this information is not always routinely available to researchers. There are also many inconsistencies between the two databases due to the different standard of data recording to identify individual animals and herds. In general, although the quality of data in the CTS database and Sam's IT system

has improved substantially over time, reducing clerical errors and developing more uniform standards for recording farm-level and animal-level data will improve the quality of these databases for future epidemiological investigations (Paiba *et al.*, 2007).

6.3.2 Model limitations

Epidemiological models are commonly used to assess the impact of alternative management strategies. The efficacy of controls is typically assumed from expert opinions rather than estimated from data. Managed endemic diseases such as bTB offer the potential to estimate the efficiency of control directly from epidemiological data. However, these models have frequently been criticised for making simplifying assumptions about the complex processes driving disease transmission dynamics (Gates, 2013). The bTB simulation model framework developed in Chapter 3 has addressed the problems of static transmission mechanism which fails to capture the animal movement dynamics by applying regular monthly cattle movements between farms in the model structure. Nevertheless, the models are still limited in assuming that all herds carry the same risk of spreading disease and that all farms have the same within-herd transmission dynamics as well as uniform contact rates and homogeneous mixing within-herd. These issues can be addressed by incorporating real cattle movement data to form realistic contact networks, while combining individual animal-level and herd-level disease data as well as demographic information to parameterise the simulation models. Furthermore, the simulation model framework can be extended to include large number of herds, and the addition of information describing farmer behaviour and herd management practices can add extra dimension to modeling the transmission dynamics and assessing the performance of surveillance activities.

Previous studies have already identified a list of potential factors that may influence the outcome of the diagnostic test for bTB (namely the SICCT and gamma-interferon test) such as concurrent infection, stages of infection and repetitive tests (de la Rua-Domenech *et al.*, 2006; Clegg *et al.*, 2011b; Strain *et al.*, 2011). The case-control studies presented in Chapters 4 and 5 further explored factors associated with physiological stress, which may also lead to suppressed immune response (de la Rua-Domenech *et al.*, 2006; Gates *et al.*, 2013). Although there was significant associations between indicators for stressful event and the diagnostic outcome from the SICCT and gamma-interferon test (i.e. movements or SICCT test within 60 days is positively associated with false negative outcome from SICCT test and recent calving is negatively associated with been identified as reactor under the SICCT and gamma-interferon), yet risk factor analyses alone is not sufficient to prove direct causation. Animal challenge study and biological experiments needs to be conducted in order to establish direct evidence of such an effect. In addition, if available, data on the SICCT and gamma-interferon test measurements (e.g. skin measurements rather than positive or negative outcome), particularly for animals that tested negative or inconclusively, may be used to quantify the potential suppression effect, thereby, allowing for adjustment for different standards (e.g. standard vs severe interpretation) when interpreting the diagnostic outcome under these circumstances.

6.4 Further work and future directions

While there are some limitations in the current analyses that will require further refinement before the outputs can be used to inform government policy, with an increasing availability of high-performance computing and detailed resolution of epidemiological data, there is almost no limit to the complexities that can be introduced into future epidemiological models. The real challenge for

veterinary researchers lies in translating the resulting scientific discoveries into practical interventions that will ultimately reduce the burden of endemic disease on cattle production systems (Gates, 2013). However, epidemiological models and data have often been criticised for using oversimplified or inappropriate assumptions and often difficult to apply in practical situations (McInerney, 1996; Stott *et al.*, 2003). Results from the analyses in this thesis provided additional evidence for the burdens of missing infections of bTB. This is partially attributable to the potential limitations in the diagnostic test protocols and the fact that scientific research outputs are rarely presented in an accessible format to help farmers and professional veterinarians to make better informed decisions to assess the disease status of their animals.

Computerised decision support systems are an important tool that can provide direct access to scientific knowledge for farmers without having to invest substantial amount of time in reviewing technical literatures (van Schaik *et al.*, 2001; Bennett *et al.*, 2012). Traditionally, these systems provide an interface that allow users to enter specific information about the farm and animal characteristics. Then, based on an underlying data-driven model, the user can explore various ‘what if’ scenarios for disease prevention and optimal control strategies. The simulation model framework developed in Chapter 3 can form the basis when designing such a decision support system for bTB, the model outputs should be realistic and capable of generating discussions amongst the veterinary professionals. Findings from the case-control studies in Chapter 4 and 5 could also be incorporated to address the problems of potential missing infections when conducting bTB diagnostic test on individual animals. The system could be used to generate warnings when testing animals that may be affected by physiological stresses or other limiting factors that have the potential to influence the diagnostic outcome. Thereby adjusting the diagnostic test interpretations (e.g. standard or severe) given the existing animal characteristics.

Despite their tremendous value, decision support systems have historically been difficult to disseminate in the cattle industry (Gates, 2013). It is easy to envision how the CTS database and the Sam's IT system could be used to improve access to the models as well as to improve the quality of their outputs. Most UK livestock keepers now use dedicated online systems to report cattle movements and results from bTB surveillance testing in order to keep up-to-date records of individual animals present on the farm as well as their previous testing history. These data could be directly downloaded into the decision support system from the online database repository and be used to generate tailored recommendations for disease control, such as the optimal surveillance strategies or the individual characteristics of animals or herds to screen as part of risk-based surveillance programmes (e.g. when to test and who to test). In any system that is used to inform real life decisions, it is also important to consider the financial burdens of implementing the model suggestions, as this can have a direct impact on farmer behaviour and management practices (Vernon, 2011; Skuce *et al.*, 2012). Similar approaches were used in agricultural economics where decision support systems were employed to help farmers to determine the financial optimal structure for their herds (Vargas *et al.*, 2001; Demeter *et al.*, 2011) and optimisation of replacement breeding cattle policies (Heikkilä *et al.*, 2008).

Finally, with the decision support system managed in a central location, the models could also be easily updated over time to reflect changes in the diagnostic tests, development in vaccinations, and effective surveillance measures used to support the disease control efforts. Thus, farmers and veterinary surgeons would have access to the latest developments in epidemiological research to make more informed decisions on herd management and disease control, which will contribute towards achieving the objective of bTB eradication in the United Kingdom by 2038, as set out by the UK government (Defra, 2014b).

6.5 Conclusion

Bovine tuberculosis continues to undermine the sustainability of modern cattle production systems despite tremendous advances in our understanding of its important epidemiological features. Overall, the key aspects of an effective bTB control programme are improved surveillance through more reliable, and possibly more frequent, testing and control measures limiting spread through the movement of cattle between herds as well as addressing infection in wildlife host to prevent sporadic re-introductions. With the increasing availability of high quality data and advances in modern technology, there are many opportunities to develop more targeted and cost-effective approaches to controlling bTB at the herd and industry levels. These efforts will require further research into the potential factors for missing infections and close collaboration with industry policy and stakeholders to ensure research outputs are delivered in an accessible and user-friendly fashion.

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